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(54) Title: GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

(57) Abstract

The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell. The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. In addition, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell. Also, the present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity. The present invention additionally provides a molecularly defined primitive hematopoietic stem cell. Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells.

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GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

GOVERNMENTAL SUPPORT

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Throughout this application, various publications are referenced by number. Full citations for these publications may be found listed at the end of the specification and preceding the Claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art. A Sequence Listing is provided.

FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cells and the stem cell and support cell genes that support stem cell replication and differentiation.

BACKGROUND OF THE INVENTION

The adult hematopoietic system is organized as a hierarchy of cells with decreasing self-renewal and multilineage differentiation potential. This is accompanied by progressively larger numbers of more mature cells and an increasing tendency to be in active cell cycle (Lemischka, I.R., 1992; Morrison, S.J., et al. 1995). Collectively, the properties of this hierarchical system result in the balanced, lifelong production of at least eight distinct cell lineages. A population of stem cells establishes the entire hierarchy; therefore, in order to understand the fundamental mechanisms responsible for normal hematopoiesis it is ultimately necessary to understand the biology of the stem cells.

Most information concerning the biology of stem cells has been obtained from the mouse model. In this system, the most critical, characteristic property of the stem cell population has been defined; that is, its ability to reconstitute a normal blood system in a transplanted host. A number of variations on the basic transplantation assay have been

described (Harrison, D.E. 1980; Spangrude, G.J., et al. 1995). All of these systems, together with the appropriate donor vs. host or clonotypic markers have rigorously defined the most primitive stem cells and have provided a description of their developmental properties. Perhaps the most striking characteristics of this cell population come from retroviral "marking" studies (Leminschka, I.R. 1992). These studies clearly show that a single stem cell clone is both necessary and sufficient, not only to sustain lifelong, multilineage hematopoiesis in one primary recipient but in numerous secondary animals. This illustrates the remarkable proliferative potential of the stem cell and directly demonstrates stem cell self-renewal. A major advance in mouse stem cell biology was the development of strategies which facilitate the substantial enrichment of stem cell activity (Bauman, J.G., et al. 1988; Spangrude, G.J., et al. 1988; Jordan C.T. , et al 1990). Purification procedures enabled the first direct approaches to unravel the mechanisms responsible for the unique biological properties of the stem cell population. A key observation was that the phenotypically defined stem/progenitor cell population is heterogeneous with respect to in vivo functional properties (Fleming, W.G., et al 1993; Li, C.L. and Johnson G.R. 1992; Spangrude, G.J. and Johnson, G.R. 1990, Jones R., et al. 1990; Uchida, N., et al. 1993). In addition to the in vivo repopulating cells, other primitive progenitor cells are often contained in a purified population (Weilbaecher, K., 1991; Trevisan, M. and Iscove, N.N. 1995; Ogawa, M. 1993). These can be assayed in a variety of in vitro systems. Whether all of these in vivo and in vitro activities represent discrete cell subpopulations or whether there is a continuum of functional potential is still an unanswered question. Recent studies have suggested distinct physical properties for functionally different activities within the primitive population Morrison, S.J., and Weissman, I.L. 1994); Morrison, S.J. et al. 1997; Jones, R.M., el al. 1996). A second set of observations revealed an inverse correlation between a tendency for active cell cycling and primitive, uncommitted developmental potential in BM (Spangrude, G.J., and Johnson, G.R. 1990). In fetal liver a higher proportion of primitive stem cells is actively cycling (Fleming, W.H., et al. 1993). Moreover, it has been shown that fetal stem cells are more potent than adult stem cells in LTRA (Jordan, C.T., et al. 1995; Pawliuk, R., et al 1996). These are exciting observations because they suggest that rapid stem cell cycling can be compatible with the maintenance of primitive in vivo activity. Very recent studies suggest that the adult BM stem cell compartment may in fact be cycling at a very slow rate (Bradford, G.B., et al. 1997) . Clearly, stem cell cycle regulation is a critical

area for investigation. Stem cell purification has facilitated studies aimed at ex vivo maintenance or expansion of the most primitive, transplantable stem cell. Most culture systems strongly favor a differentiation process (Van der Sluijs, J.P., et al. 1993; Traycoff, C.M., et al. 1996; Peters, S.O., et al. 1995; Knobel, K.M., et al. 1994). However, several recent reports have been encouraging. It has been shown that colonies grown in defined cytokines can retain not only myeloid and erythroid but also lymphoid potentials (Ball, T.C., et al. 1995). Moreover, the short-term (2-3 weeks) maintenance of LTRA has been demonstrated in suspension cultures supported by IL6, IL11, together with ckit ligand (KL) or flk2/flt3 ligand (FL) (Yonemura, Y.H., et al. 1997). A recent report has shown that colonies initiated in cytokine-supplemented semisolid cultures retain LTRA (Trevisan, M., et al. 1996). The studies described herein have developed a stromal cell line supported system which quantitatively maintains LTRA for an extended (4-7 weeks) time (Moore, F.A., et al. 1997).

In the human system it is clearly not possible to do the same kind of extensive marking and transplantation assays. However, several xenograft model systems have been developed to assess the in vivo behavior of human stem cells (Traycoff, C., et al. 1994; Turner, C., et al. 1996; Cashman, J., et al. 1997) . Some of these experiments can be done quantitatively in limiting dilution (Bhatia, M., et al. 1997). A very recent study has demonstrated a common proviral integration site in granulocyte macrophages and T-cells derived from beige/nude/XID mice 7-11 months after engraftment with genetically transduced human stem cells (Nolta, J.A., et al. 1996) . This important study paves the way for precise in vivo clonal analyses. The largest amount of functional information about human stem/progenitor cells has been obtained in vitro using a wide range of stromal cell and cytokine supported culture systems. It is not possible herein to describe and properly accredit all of the important studies, however several advances deserve mention. The long-term culture-initiating cell (LTCIC) assay measures the in vitro production of colony forming cells (CFC) after periods of at least five weeks in culture (Sutherland, H.S., et al. 1989) . The cells producing these CFC derive from a population of cells which, at least to some extent, probably overlaps with the most primitive compartment. The maintenance and expansion of primitive functional abilities in this culture system has recently been documented (Petzer, A.L., et al. 1996) . A variation on this assay system, the extended LTCIC (ELTCIC) has been suggested to measure an even more primitive cell population in BM and CB (Hao, Q.L., et al. 1996) . A very exciting

prospect for the near future will be the integration of the various in vivo xenograft assays with the in vitro LTCIC and ELTCIC systems. Some very recent efforts have suggested that the NODSCID xenograft system and the LTCIC assay may measure distinct stem/progenitor subsets (Larochelle, A..J., et al. 1997). Clearly, much more work needs to be done, however, it may be anticipated that the ELTCIC system will provide the "bridge" in this continuum. Collectively, and including the various strictly cytokine-driven systems, the above studies illustrate the current possibility to accurately and quantitatively reveal the majority (if not all) functional entities in the human stem/progenitor cell hierarchy. The physical characterization and purification of human stem/progenitor cells has proceeded along lines which are parallel to the mouse system. Indeed, because of clinical impetus, it can be argued that they are further advanced. Thus, as measured in the range of assays discussed above, the consensus physical phenotype of the most primitive portion of the human stem/progenitor hierarchy is CD34+Lin-CD38- (Terstappen, L.W.M.M., et al. 1991). The CD34+Lin-CD38+ subset contains less primitive, more committed cellular entities. Other studies have shown that, similar to the mouse, low level expression of Thy1 (CD90) is a feature of the primitive human stem cell (CD34+Lin-CD90+)(Baum, C.M., et al. 1992; Craig, W., et al. 1993). Most CD90+ cells in this compartment are CD38-. Therefore, the consensus phenotype can be described as CD34+Lin-CD90+ (Craig, W., et al. 1993). Two potential differences with the murine system can now be highlighted. First, a very recent and elegant study has shown that the most primitive mouse stem cell may in fact be CD34-/lo (Osawa, M., et al. 1996) . Whether this is a genuine difference or whether it reflects the ability to perform more accurate long-term engraftment studies in the mouse remains to be determined. Second, it has been suggested that in the mouse, CD38 expression is a positive indicator for primitive stem cell function in a purified population (Randall, T.D., et al. 1996) . As in the mouse, human stem/progenitor cells have been identified and purified from various sources. These include: adult BM (Baum, C.M., et al. 1992), CB (DiGiusto, D.L., et al 1996) , fetal liver (Craig, W., et al. 1993) and peripheral blood stem cells after various mobilization protocols (Murray, L., et al. 1994). Similar to the data obtained in the murine system, comparative studies reveal that, in general, the basic and fundamental functional properties of stem/progenitor cells are shared regardless of the tissue source. There are however, significant functional and physical differences.

Numerous insights into hematopoietic molecular control mechanisms have come from gene-targeting studies in mice. Mutations in specific genes, most notably, those encoding transcription or DNA-binding proteins, have profound cell-intrinsic, global or lineage-specific effects on hematopoietic development (Shivdasani, R.A., and Orkin, S.H. 1996; Orkin, S.H. 1996). In the latter cases, it is tempting to speculate that the phenotypes result from defects in the commitment process. However, malfunctions in the commitment decision to "set up" a program of differentiation are difficult to distinguish from malfunctions in the differentiation program itself. Two gene products, AML1 (CBF2) and SCL (tal-1) appear to be necessary global regulators of hematopoiesis (Wang, W., et al. 1996; Okuda, T., et al. 1996; Porcher, C., et al. 1996; Robb, L. , et al. 1996) . Whether these molecules act to specify a hematopoietic stem cell or by other means is an open question. Interestingly, both of these molecules play roles in leukemic transformation. A very important gain of function study documents the apparent ability of HOXB4 to increase primitive cell numbers without significant impairment of differentiation abilities (Sauvageau, G., et al. 1995) . Together with observations that HOXA9 is translocated in myeloid leukemia (Nakamura, T., et al. 1996; Borrow, J., et al. 1996) , these studies suggest an important hematopoietic role for homeobox proteins. Without question, the above and other studies have identified important regulators of hematopoiesis. However, in almost all cases these regulators were first identified in other systems. The opposite approach is to directly search for stem cell regulators in stem cells, Graf, L., and Torok-Storb, B. 1995; Yang, Y., et al. 1996). The present invention solves these problems.

SUMMARY OF THE INVENTION

The human hematopoietic stem/progenitor cell population has been extensively characterized according to physical and antigenic criteria as well as in a variety of in vitro and in vivo assay systems. Collectively the human studies have revealed similarities to the hierarchical stem/progenitor cell organization defined in the murine system. In spite of significant strides in the identification of cytokines which can act on stem cells, it has not been possible to define a system where undifferentiated expansion of the most primitive stem cell population occurs. Similarly, it has not been possible to direct differentiation along lineage-specific pathways. These limitations, which also apply to the murine system, have hampered the elucidation of regulatory mechanisms which

mediate the most fundamental aspect of stem cell biology; that is, the decision to self-renew or commit to differentiation. As a consequence, very little is known about the molecular biology of the most primitive hematopoietic stem cell in any organism. It was hypothesized that the regulation of primitive stem cells will be mediated at least in part by the products of genes which are uniquely or predominantly expressed in these cells. One precedent for an important, differentially-expressed molecule is the flk2/flt3 receptor tyrosine kinase. There presumably are other important and differentially expressed gene products. Therefore, it is an object of the present invention to identify these molecules and address their functional roles. Specifically, an aspect of identification of gene expression patterns specific to primitive human stem cells is the molecular phenotype of the human stem cell. The present invention describes methods to define the profile of genes specifically expressed in undifferentiated human stem/progenitor cell populations.

A primary focus of the present invention is on primitive cells isolated from normal bone marrow (BM) samples. The present invention further comprehends use of other sources of stem cells, such as umbilical cord blood (CB).

The methods of the present invention combine diverse technical approaches and sophisticated bioinformatic analyses.

This invention further provides methods to identify genes whose expression can be modulated by cytokine or stromal-dependent culture and/or by cell-cycle status.

Another object of the present invention is to provide methods for the functional characterization of human stem cell-specific gene products. An aspect of this invention is a method to facilitate the functional characterization of specifically expressed gene products as candidate regulators of a variety of stem/progenitor cell processes. In particular, a provided method uses an in vitro system which approximates many characteristic properties of normal stem cells to analyze positive and negative regulation of proliferation, cell-cycle parameters, apoptosis and commitment.

It is a further goal of the present invention to provide a necessary (and usually missing) component for stem cell gene-expression screens; that is, the ability to quickly assess the function of extensive panels of genes.

It is also an object of the present invention to provide a method for the functional identification of stem cell regulators. An aspect of this invention is a facile screening method for "categorizing" large populations of specifically-expressed molecules according to their potential roles in a variety of stem/progenitor cell processes. Gain of

function as well as loss of function approaches are contemplate by the present invention. This method does not rely on any prior knowledge of nucleotide or predicted protein sequence.

Accordingly, the present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics: (1) specifically expressed in the hematopoietic stem cell; and (2)

encoding a hematopoietic stem cell – specific protein.

The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. The present invention further still provides a nucleic acid probe capable of specifically hybridizing with the provided nucleic acid under standard hybridization conditions.

Also, the present invention provides an antibody capable of specifically binding to the provided protein without substantially cross-reacting with a non-stem cell specific protein or homologs thereof under conditions permissive to antibody binding. Additionally, the present invention provides a cell capable of producing the provided antibody.

In addition, the present invention provides a method for identifying the presence of a primitive hemopoietic stem cell in a sample comprising nucleic acids specifically expressed in hematopoietic stem cells. Further still, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample.

The present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity.

The present invention even further still provides a method for identifying primitive hematopoietic stem cell-specific nucleic acids.

Also the present invention additionally provides a molecularly defined primitive hematopoietic stem cell.

Yet additionally, the present invention provides a method for treating a condition in a subject comprising administering to the subject a therapeutically effective amount of a provided pharmaceutical composition. The present invention provides a method of introducing an exogenous nucleic acid into a hematopoietic stem cell.

Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells. The expanded cells are available to receive exogenous genes, including by retroviral or other vectors which require a round of replication. Alternatively, the cells are available for transplantation either autologously or heterologously.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1. Schematic "flow of information" strategy.

FIGURE 2. Mammalian stem cell system (black circle = strong hybridization signal, gray circle = detectable hybridization signal, and open circle = no detectable hybridization signal). The Smc-34 cDNA is a completely novel sequence with a predicted leucine zipper and several potential membrane spanning domains.

FIGURES 3A-3B. (right panel), the control, non-subtracted RDA cDNA population (38-) contains b-actin sequences which are missing in the two subtracted RDA populations, 38- and 38-)38+. A differentially expressed gene (HDD-2, described below) is enriched in the 38- RDA population and at least retained in the 38-)38+ RDA population (Figure 3A, left panel). Two, bi-directional, RDA cDNA populations (38- and the converse 34-)38) were used to probe (See Figure 3) duplicate arrays of a subtracted 38- library (Figure 3B).

FIGURE 4A-4E. **Figure 4A:** 34B4 (SEQ.ID.No.: 69) is closely related to a gene encoding TINUR The sequence homologies and restricted expression pattern of 34A5 is shown in **Figures 4B and 4C**. In **Figure 4C and 4D** (and also 5a, 5b, and 6b) there are twenty-one samples of capfinder-amplified cDNA from various hematopoietic populations. From left to right these are: four CD34+Lin- populations, three CD34+Lin-CD90+ populations, two CD34- populations, four CD34+Lin-CD38+ (obtained from the same BMs as the CD38- samples in lanes 1-4), two CD34+Lin- samples (obtained from the same BMs as the CD90+ samples in lanes 5 and 6), three CD34+Lin- populations obtained after 1, 2 or 4 days of culture and finally their three CD34+Lin-CD38+ counterparts. The 38G2 cDNA (SEQ.ID.No.: 70) is closely homologous to the

LTG9/MLLT3 gene located on 9q22 and involved in t(9;11) leukemia (Iida, S., et al. 1993) (**Figure 4E**).

FIGURES 5A-5B. G0S3, a fos-related gene (Heximer, S., et al. 1996) (**Figure 5A**) and (2) HLA-DR (**Figure 5B**).

FIGURES 6A-6D. **Figure 6A.** HDD-2 is about 500 bp. It contains a short open reading frame of 89 amino acids (SEQ.ID.No.: 71). The predicted peptide sequence is shown. **Figure 6B**. The expression profile of HDD-2 demonstrates that it is stem cell restricted. **Figure 6C.** HDD-2 hybridization to a dot blot with numerous human pA+ mRNA samples (Clontech). HDD-2 hybridization is only visible in kidney (the other "spots" are background). **Figure 6D.** Genomic Southern blot confirmed that HDD-2 corresponds to a single-copy human gene.

FIGURE 7. Immunoprecipitation analysis of protein extracts using rat IgG2b isotype control antibody (IgG) or AA4.1 mAb. Protein extracts were prep-ared from D2N cells; M2.4 cells; AA4-depleted fetal liver cells (FLAA4-); AA4enriched fetal liver cells (FLAA4+); AA4-depleted bone marrow cells (BMAA4-); AA4-enriched bone marrow cells (BMAA4+). Indicated on the right are positions of molecular weight markers.

FIGURES 8A-8C. AA4 expression in retrovirus infected cells. Figure 8A. Flow cytometry analysis of NIH 3T3 and EML Cl cells using PE-conjugated AA4.1 antibody before and after infection with REBNA/AA4. Figure 8B. Immunoprecipitation of biotin-labeled surface proteins using rat IgG2b isotype control antibody (lanes 1, 3, 5) or AA4.1 mAb (lanes 2, 4, 6). Cellular extracts were prepared from D2N cells (lanes 1 and 2), REBNA/AA4 infected NIH 3T3 fibroblasts (lanes 3 and 4), and REBNA/AA4 infected EML Cl cells (lanes 5 and 6). Figure 8C. Immunoprecipitation of cellular extracts using AA4.1. REBNA/AA4 NIH 3T3 cells (lanes 2 -- 4) and REBNA/AA4 EML Cl cells (lanes 6 -- 8) were labeled with 35S-methionine and chased with nonradioactive media for 10 min. (lanes 3 and 7) or 20 min. (lanes 4 and 8). REBNA/GFP NIH 3T3 (lane 1) and REBNA/GFP EML Cl cells (lane 5) are shown as controls. Indicated on the right are positions of molecular weight markers.

FIGURE 9. Nucleotide sequence (SEQ.ID.No.: 72) and the deduced amino acid sequence of AA4 (SEQ.ID.No.: 73). Amino acid sequences is numbered 1 with initiator methionine. Underlined is the putative signal peptide in AA4. The transmembrane domain is double underlined. Light grey line indicates C-type lectin carbohydrate recognition domain. Dark grey lines show EGF-like repeats, while broken grey lines indicate calcium-binding EGF motifs.

FIGURES 10A-10B. Comparison of the primary structures of AA4 and human ClqR. **Figure 10A.** Alignment of amino acid sequences of AA4 (SEQ.ID.No.: 73) and ClqR. **Figure 10B.** Comparison of the domain structures of AA4 and ClqR. The proteins contain N-terminal signal peptides (SP), a C-type lectin recognition domain, six EGF-like domains, including three calcium-binding (cb) EGF-like domains, and a transmembrane domain (TM).

FIGURE 11. In normal tissues and transformed cell lines, a 7kb RNA species hybridizes with the cloned cDNA (Figures 11A and 11B). In addition to the 7kb species, poly(A)-RNA from D2N cells contains a minor band corresponding to a 3.2 kb mRNA (Figure 11A, lane 8).

FIGURES 12A-12B. Northern blot analysis of AA4 expression in transformed cell lines **Figure 12A** and normal mouse tissues **Figure 12B.** Indicated on the left are positions of 9.44 - 0.24 kb RNA molecular weight markers. Hybridization with D2N poly(A)+ RNA is shown after a 2 hr and overnight exposures. Hybridizations with GAPDH are shown as controls for equal loading.

FIGURES 13A-13C. RT-PCR of cDNAs prepared from murine fetal liver, (A) bone marrow-derived hematopoietic cells (B), or differentiating ES cells (C). **Figure 13A.** Lanes 1 and 2, AA4- cells; lane 3, AA4+ cells; lane 4, AA4+Sca-l+c-Kit+LinlO cells; lane 5, AA4+Sca-l-c-Kit+LinIO cells. **Figure 13B.** Lane 1, RholOSca-l+Thy-11OLincells; lane 2, RhohiSca-l+Thy-11OLin- cells; lane 3, Sca-l+Thy-l-Lin- cells; lane 4, Lin+ cells. **Figure 13C.** ESO, undifferentiated ES cells; BL1 and 2, blast cell colonies; ENT1 and 2, differentiated endothelial cells; HMT1, 2 and 3, differentiated hematopoietic cells. The cDNAs probes used for hybridization are indicated on the right.

FIGURE 14. AFT024 maintains *in vivo* repopulating stem cells. The ability of 3 different stromal cell lines to support highly purified fetal liver stem cells was studied. Freshly purified day 14 fetal liver cells were transplanted directly (10^3 AA4.1$^+$, lin$^{-/lo}$, Sca-1$^+$, c-kit$^+$ cells plus 10^6 Ly5.1 competitor BM per mouse; n=6) into Ly5.1 congenic mice (Control). Additional stem cells from the same purification were also used to initiate Dexter-LTC over irradiated AFT024, 2012, and 2018 stromal cell monolayers (10^4 cells/10 cm dish). After 4 weeks of culture, 10% aliquots of each culture (10^3 stem cell equivalents) were transplanted into Ly5.1 recipients (n=8) together with 10^6 competitor Ly5.1 BM cells. Peripheral blood cells from mice were analyzed for the presence of Ly5.2$^+$ cells at 5, 12, 24, and 56 weeks after transplant. Error bars represent SEM.

FIGURE 15. Long-term culture on AFT024 maintains greater levels of repopulating stem cell activity than short-term cytokine- or short-term AFT024-supported cultures. The levels of stem cell activity maintained in short-term cytokine-supported and short-term AFT024-supported cultures were compared to those maintained in long-term AFT024 coculture. Purified fetal liver cells were cultured for 5 days in suspension with cytokines or on an AFT024 monolayer (3000/well-12-well tray). Additional cells from the same purification were seeded onto AFT024 monolayers (3000/6 cm dish) and maintained in Dexter-LTC for 5 weeks. At completion of both culture periods the cells were harvested, mixed with Ly5.1 BM and used to transplant mice. Each mouse received 20% of each culture (600 stem cell equivalents) and $4X10^5$ competitor BM cells (4 mice/culture). Peripheral blood cells from mice were analyzed for the presence of Ly5.2$^+$ cells at 15 weeks after transplant. FL 1.0±0.57; FL/SL 0.75±0.25; FL/IL-6 1.8±0.14; SL/IL-6 3.2±0.46; FL/IL-6/SL 1.7±0.21; AFT024 5 days 2.8±0.11; AFT024 5 weeks 32.2±7.4 * ($p<0.004$) Students t-test. See Methods for culture conditions and cytokine concentrations. FL, flk2/flt3-ligand; SL, steel factor. Error bars represent SEM.

FIGURE 16. Time course of cobblestone area formation on AFT024. The formation of stromal dependent CA derived from purified fetal liver stem cells was studied in AFT024 cocultures. Characteristic clusters of at least 50 cells were scored as CA over 28 days of culture. Results are expressed as the mean number of CA/1000 input stem cells from 3

separate fetal liver purifications (300-600 cells/well in 12-well trays). Error bars represent the SEM. The frequency of CA after 28 days is approximately 1 for every 20 input stem cells.

FIGURE 17. High-proliferative potential multilineage clonogenic progenitors are selectively expanded on AFT024. The clonogenic progenitor content of stem cells maintained in AFT024 supported Dexter-LTC was determined. Enriched fetal liver stem cells were seeded onto AFT024 monolayers, at various time points, an individual well was harvested and the cells placed into semi-solid clonogenic progenitor assay (CFU-C). Colonies were scored at 8-14 days. Colonies were designated as HPP upon reaching a size $\geq$1 mm after 8 days. CFU numbers at days 0, 4, and 28 are averaged from 3-5 individual stem cell purifications. Error bars represent the SEM for these experiments. Other time points are individual determinations. CFU are normalized to 1000 input stem cells in the stromal cocultures for comparison to day 0 progenitors. *CFU-Mix (p=.01) and *CFU-HPP-Mix (p=.001) are significantly expanded at day 28 compared to day 0, (Student's T-test).

FIGURES 18A-18B. Cobblestone area-initiating cells are expanded on AFT024. **Figure 18A.** A quantitative estimate of the number of 28 day cultured stem cell equivalents required to form a CA after replating on secondary AFT024 monolayers was determined. Four different AFT024 cocultures from separate fetal liver purifications were studied (A, B, C, and D). In limiting-dilution assay, the frequency of stem cell equivalents required to form a CA in another 7 days was 1 in 4 (3.56 ± 0.64, $r^2=0.96$). **Figure 18 B.** CA maintenance in one of the 4 above cultures was followed for an additional 4 weeks. The frequency of stem cell equivalents maintaining CA was determined. At 37% negative wells the frequencies were: 2 weeks 1 in 3, 3 weeks 1 in 10, 4 weeks 1 in 19, and at 5 weeks 1 in 29.

FIGURES 19A-19B. Dlk expression analysis in stromal cell lines. **Figure 19A.** (*Top*) A 1.6 kb dlk transcript is visualized in the parental AFT024 and 2012 cell lines and their subclones, but not in 2018, CFC034 and BFC012. (*Bottom*) The same filter hybridized with a b-actin probe. **Figure 19B.** RT-PCR analysis of 14 fetal liver-derived stromal cell lines and other cell lines.

FIGURE 20. Cobblestone area formation by hematopoietic stem cells in the presence of soluble dlk protein. Data are from 4 experiments; 2 each with adult BM cells (Sca-1^+, c-*kit*$^+$, lin$^{lo/-}$) and day 14 fetal liver cells (AA4.1^+, Sca-1^+, c-*kit*$^+$, lin$^{lo/-}$). Results are expressed as the ratio/fold-increase in CSA number for fourteen data points each (bars represent, none vs control; dlk vs none; dlk vs control) for the 4 different experiments. Error bars represent SEM. *P= 0.01 comparing dlk vs none to none vs control, **P= 0.001 comparing dlk vs control to none vs control (Student's t-test).

FIGURE 21. Membrane bound dlk expression in transfected BFC012 cells. Full length dlk cDNA was transfected into BFC012 cells. (*Left*) A flow diagram of dlk expression in transfected BFC012 populations (BFC-dlk) and cells transfected with the selection plasmid alone (BFC-Zeo). (*Right*) Expression of dlk in a cloned line (BFC-dlk-5) derived from the expressing population and a control clone (BFC-Zeo-1).

FIGURES 22A. CSA formation by hematopoietic stem cells in the presence of membrane-bound dlk. **Figure 22A.** Bars labeled BFC are from 5 groups (nontransfected BFC012 cells, two control pZeo transfected BFC012 populations, and two clones derived from the pZeo transfected populations). Bars labeled BFC-dlk are from three groups shown to express transfected dlk; one dlk-transfected BFC012 population (BFC-dlk) and two individual transfected clones (BFC-dlk-1 and BFC-dlk-5). Error bars represent SEM. ** P< 0.001 days 3, 4, and 5; *P< 0.01 days 6 and 7 (Student's t-test). **Figure 22B.** A clone derived from the dlk transfected populations of BFC012 cells (BFC-dlk-5) and a clone derived from pZeo transfected populations (BFC-Zeo-1) were used for CSA assay with purified fetal liver stem cells. CSA/1000 input stem cells are expressed as the mean of three individual experiments, error bars represent the SEM. **P< 0.001 at days 4, 6, and 8 (Student's t-test).

FIGURES 23A-23B. HPP multilineage clonogenic progenitors and *in vivo* repopulating stem cells are maintained in short-term dlk-expressing cocultures. **Figure 23A.** Fetal liver stem cells were purified as described and assayed for their progenitor content immediately after purification and after culture on BFC-dlk-5, and BFC-Zeo-1. At day 4 the cultures were used for clonogenic progenitor (3 experiments) and transplantation assay (2 experiments). Bars represent data from 3 experiments with day 0 cells (Fresh) and day 4

cocultured cells (BFC-dlk-5 and BFC-Zeo-1), error bars represent SEM. *P= 0.01 for total CFU-C from fresh stem cells compared to total CFU-C from BFC-dlk-5 cocultures at day 4; ** P= 0.001 for total CFU-C from BFC-dlk-5 compared to total CFU-C from BFC-Zeo-1 (Student's t-test). **Figure 23 B.** Analysis of *in vivo* repopulating ability of purified fetal liver stem cells cocultured for 4 days on BFC-dlk-5, BFC-Zeo-1, and AFT024 monolayers. Results are from nine individual mice in two experiments (4-5 mice in each experiment) at 10 weeks after transplantation. P= 0.05 for BFC-dlk-5 vs BFC-Zeo-1 (Student's t-test).

FIGURE 24. Genes and predicted proteins isolated from primitive stem cells by the techniques of the present invention. (Sequence Identification Numbers are indicated on the figure).

DETAILED DESCRIPTION

The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics: (1) specifically expressed in the hematopoietic stem cell; and (2) encoding a hematopoietic stem cell – specific protein.

An embodiment of this invention further comprises the following characteristic: capable of hybridizing under standard conditions with a sequence selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. A portion thereof, in a preferred embodiment of this invention is the 5' end region or the 3' end region of the nucleic acid.

In another preferred embodiment, a portion thereof is at least a 8-18 nucleotide portion of the coding region. In yet another preferred embodiment, a portion therof is at least a 8-18 nucleotide portion of a non-coding regulatory region or a binding region such as a stem-cell specific promoter or enhancer region. According to still another embodiment of the provided isolated nucleic acid further comprises the characteristic of encoding a protein capable of modulating hematopoietic stem cell activity. According to this invention modulating hematopoietic stem cell activity includes up-regulating, down-regulating or otherwise changing the activity of the hematopoietic stem cell. Such activity is contemplated as inducing differentiation or inhibiting differentiation of the cell. However, directing differentiation toward one or another daughter cell type is also within the scope of a preferred embodiment of this invention. Other preferred embodiments include but are not limited to modulation of transcription, translation, gene splicing, transport, proteolytic processing, replication, expression of cell surface markers and transplantation. According to still another embodiment of the present invention, the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication. According to yet another embodiment of this invention, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. In one preferred embodiment of this invention, the provided nucleic acid comprises the nucleotide sequence of SEQ.ID.No.: 72, an analog thereof, or a portion thereof. According to another preferred embodiment of this invention, the hematopoietic stem cell is a primitive hematopoietic stem cell. In one embodiment of this invention, the primitive hematopoietic stem cell is selected from the group consisting of an umbilical cord cell , a bone marrow cell and a fetal liver cell. In a preferred embodiment of this invention, the primitive hematopoietic stem cell is selected from the group consisting of a AFT024 cell, a 2012 cell and a 2018 cell.

The present invention further provides a composition comprising the provided nucleic acid, wherein the nucleic acid comprises one selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25,

SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. According to one embodiment of this invention, the the nucleic acid is selected from the group consisting of DNA, RNA and cDNA. Another embodiment of this invention is a vector comprising the provided nucleic acid. According to yet another embodiment, the vector comprises viral or plasmid DNA. A further embodiment of this invention is an expression vector comprising the provided nucleic acid and a regulatory element. A still further embodiment of this invention is a host vector system which comprises the expression vector in a suitable host. In a preferred embodiment of this invention, the suitable host is selected from the group consisting of a bacterial cell, a eukaryotic cell, a mammalian cell and an insect cell.

The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. According to one embodiment of this invention is the provided protein further comprising the characteristic of being capable of modulating hematopoietic stem cell activity. According to this invention modulating hematopoietic stem cell activity includes up-regulating, down-regulating or otherwise changing the activity of the hematopoietic stem cell. Such activity is contemplated as inducing differentiation or inhibiting differentiation of the cell. However, directing differentiation toward one or another daughter cell type is also within the scope of a preferred embodiment of this invention. Other preferred embodiments include but are not limited to modulation of transcription, translation, gene splicing, transport, proteolytic processing, replication, expression of cell surface markers and transplantation. According to still another embodiment of the present invention, the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication. According to yet another embodiment of this invention, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. According to one embodiment the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic

stem cell replication. According to another embodiment, the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor. According to still another embodiment, the protein has substantially the same amino acid sequence as one selected from the group consisting of SEQ.ID.No.: 42, SEQ.ID.No.: 44, SEQ.ID.No.: 46, SEQ.ID.No.: 48, SEQ.ID.No.: 50, SEQ.ID.No.: 52, SEQ.ID.No.: 54, SEQ.ID.No.: 56, SEQ.ID.No.: 58, SEQ.ID.No.: 60, SEQ.ID.No.: 62, SEQ.ID.No.: 64, SEQ.ID.No.: 66, SEQ.ID.No.: 68, SEQ.ID.No.: 70, SEQ.ID.No.: 71, and SEQ.ID.No.: 73.

The present invention further still provides a nucleic acid probe capable of specifically hybridizing with the provided nucleic acid under standard hybridization conditions.

Also, the present invention provides an antibody capable of specifically binding to the provided protein without substantially cross-reacting with a non-stem cell specific protein or homologs thereof under conditions permissive to antibody binding. Additionally, the present invention provides a cell capable of producing the provided antibody.

In addition, the present invention provides a method for identifying the presence of a primitive hemopoietic stem cell in a sample comprising nucleic acids specifically expressed in hematopoietic stem cells comprising (a) contacting the sample with the provided antibody under conditions permissive to the formation of an antibody complex; (b) detecting the presence of the complex formed in step (a), the presence of a complex formed indicating the presence of a primitive hemopoietic stem cell in the sample. According to one embodiment of this invention, the antibody is labeled with a detectable marker. In a preferred embodiment, the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, magnetic bead, dye, flourescent marker and biotin.

Further still, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the provided protein . Another embodiment of this invention, provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the provided nucleic acid. According to an preferred embodiment, the nucleic acid is in an expression vector. According to another preferred embodiment the nucleic acid is introduced into the cell under conditions

permissive to the expression of the nucleic acid.

The present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity comprising: (a) contacting the hematopoietic stem cell with the sample; (b) determining the hematopoietic stem cell activity; and (c) comparing the hematopoietic stem cell activity determined in step (b) with the activity determined in the absence of the compound an increase or decrease in hematopoietic stem cell activity indicating the presence in the sample of a compound that modulates hematopoietic stem cell activity. According to one embodiment, the activity is selected from the group consisting of gene expression, replication, differentiation, transplantation, and self regeneration. The present invention also still further provides a compound identified by the method of this invention, previously unknown.

The present invention even further still provides a method for identifying primitive hematopoietic stem cell-specific nucleic acids , comprising: (a) creating a primitive hematopoietic stem cell cDNA library and a non-primitive stem cell immune cell cDNA library; and (b) subtracting the two libraries, thereby identifying primitive stem cell specific nucleic acids. According to one embodiment is (i) contacting the nucleic acids of the stem cell and non-stem cell libraries with each other under conditions permissive to hybridization, thereby forming hybrid complexes; (ii) separating the hybrid complexes formed in step (b) from the nucleic acids which did not form complexes; and (iii) isolating the nucleic acids which did not form complexes, thereby identifying hematopoietic stem cell specific nucleic acids. In still another embodiment, step (ii) further comprising amplification of the nucleic acids. Yet another embodiment is step (iii) further comprising ampliciation of the nucleic acids which did not form complexes. Even still another embodiment is further comprising displaying the amplified DNA on a chromatography gel. A further embodiment is step (b) comprising differential display of the two libraries, thereby identifying primitive stem cell specific nucleic acids. Also yet another embodiment is step (b) comprising representation difference analysis of the two libraries, thereby identifying primitive stem cell specific nucleic acids. Yet even another embodiment is further comprising cloning the stem cell specific nucleic acid. According to a preferred embodiment, the stem cell is selected from the group consisting of AF024, 2012, and 2018. The present invention further provides a nucleic acid identified by the provided method.

The present invention additionally provides a composition comprising the provided compound and a carrier. An embodiment of this invention is a pharmaceutical composition comprising the provided compound and a pharmaceutically acceptable carrier.

Also the present invention additionally provides a primitive hematopoietic stem cell specifically expressing one selected from the group consisting of: SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof. An embodiment of this invention is a primitive hematopoietic stem cell specifically expressing a nucleic acid identified by the provided method.

Yet additionally, the present invention provides a method for treating a condition in a subject comprising administering to the subject a therapeutically effective amount of the provided pharmaceutical composition. According to an embodiment of this invention, the condition is an immune system condition. In a further embodiment of this invention, the condition is leukemia.

The present invention provides a method of introducing an exogenous nucleic acid into a hematopoietic stem cell comprising contacting the stem cell with the provided composition.

Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells comprising contacting the cell with the provided composition. According to an embodiment of this invention, the ex vivo expanded hematopoietic stem cells are available for therapeutic use. The expanded cells are available to receive exogenous genes, including by retroviral or other vectors which require a round of

replication. Alternatively, the cells are available for transplantation either autologously or heterologously.

As used herein, the term, a sequence is conserved if there is substantial homology of sequence between multiple gene species.

As used herein, the terms, "hybridization" and "binding" in the context of probes, primers and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of complementarity, the length of the probe, and the stringency of the binding conditions. The higher the stringency, the higher must the degree of complementarity, and/or the longer the probe.

As used herein, the term, probe, refers to an oligonucleotide designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed, in relation to its length, to be bound under selected stringency conditions. Primers may vary in length. Preferably such primers should be sufficiently long to hybridize to the modified RNAs in a specific and stable manner. A semi-random primer as the term is used herein, encompasses a class of primers wherein either a discrete portion of the primer is random, while another discrete portion is conserved as well as primers which have nucleotide preferences at particular positions within a sequence. For example, the discrete portion-type primer may have a predetermined adaptor sequence at its 5' end and a random sequence at its 3' end. Alternatively, several preferred primers have nucleotide preferences at specific positions within the primers while other positions are random. A degenerate primer as the term is used herein, encompasses a cocktail or mixture of primers wherein one or more of the possible triplet nucleotide sequences encoding an amino acid is incorporated into the primer sequence. For example, Serine may be encoded by six separate triple sequences (AGU, AGC, UCU, UCC, UCA, and UCG). Thus, a "degenerate" primer may reflect the degeneracy of the nucleotide triplet code. Alternatively, a randomized primer, as the term is used herein, encompasses a primer wherein, the nucleotide at one or more positions may be randomized in order to yield a triplet sequence encoding an alternative or a random amino acid at the position.

An end region as the term is used herein, consists of the end nucleotide and a portion of the region including as much as that half of the entire sequence. For example, the "3' end region" or "3' region" of a primer may include the 3' half of the primer.

A preferred method of hybridization is blot hybridization. See Sambrook et al. 1989 *Molecular Cloning: A Laboratory Manual* 2nd Ed. for additional details regarding blot hybridization. Using this method, separated amplification products are transferred onto a solid matrix, such as a filter. The probe, which is detectable, either directly or indirectly, is hybridized to the solid matrix under appropriate conditions. The matrix is washed to remove excess probe. Thereafter the probe which specifically hybridized to the solid matrix can be detected.

The probe can be DNA or RNA and can be made detectable by any of the many labeling techniques readily available and known to the skilled artisan. Such methods include, but are not limited to, radio-labeling, digoxygenin-labeling, and biotin-labeling. A well-known method of labeling DNA is ^{32}P using DNA polymerase, Klenow enzyme or polynucleotide kinase. In addition, there are known non-radioactive techniques for signal amplification including methods for attaching chemical moieties to pyrimidine and purine rings (Dale, R.N.K. et al, 1973 *Proc. Natl. Acad. Sci. USA* 70:2238-42), methods which allow detection by chemiluminescence (Barton, S.K. et al, 1992 *J. Am. Chem. Soc.* 114:8736-40) and methods utilizing biotinylated nucleic acid probes (Johnson, T.K. et al, 1983 *Anal. Biochem.* 133:125-131; Erickson, P.F. et al, 1982 *J. Immunol. Methods* 51:241-49; Matthaei, F.S. et al, 1986 *Anal. Biochem.* 157-123-28) and methods which allow detection by fluorescence using commercially available products. Non-radioactive labeling kits are also commercially available.

A basic description of nucleic acid amplification is described in Mullis, U.S. Patent No. 4,683,202, which is incorporated herein by reference. The amplification reaction uses a template nucleic acid contained in a sample, two primer sequences and inducing agents. The extension product of one primer when hybridized to the second primer becomes a template for the production of a complementary extension product and vice versa, and the process is repeated as often as is necessary to produce a detectable amount of the sequence.

The inducing agent may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E.coli* DNA polymerase I, thermostable *Taq* DNA polymerase, Klenow fragment of *E.coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, reverse transcriptase and other enzymes which will facilitate combination of the nucleotides in the proper manner to form

amplification products. The oligonucleotide primers can be synthesized by automated instruments sold by a variety of manufacturers or can be commercially prepared based upon the nucleic acid sequence of this invention.

The degree of hybridization depends on the degree of complementarity, the length of the nucleic acid molecules being hybridized, and the stringency of the conditions in a reaction mixture. Stringency conditions are affected by a variety of factors including, but not limited to temperature, salt concentration, concentration of the nucleic acids, length of the nucleic acids, sequence of the nucleic acids and viscosity of the reaction mixture. More stringent conditions require greater complementarity between the nucleic acids in order to achieve effective hybridization.

"Hybridization" and "binding" in the context of probes, primers and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of complementarity, the length of the probe, and the stringency of the binding conditions. The higher the stringency, the higher must the degree of complementarity, and/or the longer the probe.

"Probe" refers to an oligonucleotide designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed, in relation to its length, to be bound under selected stringency conditions.

Primers may vary in length. Preferably such primers should be sufficiently long to hybridize to the modified RNAs in a specific and stable manner.

A semi-random primer as the term is used herein, encompasses a class of primers wherein either a discrete portion of the primer is random, while another discrete portion is conserved as well as primers which have nucleotide preferences at particular positions within a sequence. For example, the discrete portion-type primer may have a predetermined adaptor sequence at its 5' end and a random sequence at its 3' end. Alternatively, several preferred primers have nucleotide preferences at specific positions within the primers while other positions are random.

A degenerate primer as the term is used herein, encompasses a cocktail or mixture of primers wherein one or more of the possible triplet nucleotide sequences encoding an amino acid is incorporated into the primer sequence. For example, Serine may be encoded by six separate triple sequences (AGU, AGC, UCU, UCC, UCA, and UCG).

Thus, a "degenerate" primer may reflect the degeneracy of the nucleotide triplet code. Alternatively, a randomized primer, as the term is used herein, encompasses a primer wherein, the nucleotide at one or more positions may be randomized in order to yield a triplet sequence encoding an alternative or a random amino acid at the position.

Solid matrices are available to the skilled artisan. Solid phases useful to serve as a matrix for the present invention include but are not limited to polystyrene, polyethylene, polypropylene, polycarbonate, or any solid plastic material in the shape of test tubes, beads, microparticles, dip-sticks, plates or the like. Additionally matrices include, but are not limited to membranes, 96-well microtiter plates, test tubes and Eppendorf tubes. Solid phases also include glass beads, glass test tubes and any other appropriate shape made of glass. A functionalized solid phase such as plastic or glass which has been modified so that the surface carries carboxyl, amino, hydrazide, or aldehyde groups can also be used. In general such matrices comprise any surface wherein a ligand-binding agent can be attached or a surface which itself provides a ligand attachment site.

As used herein, "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. A pharmaceutically acceptable carrier encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, water emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. The invention also provides for pharmaceutical compositions together with suitable diluents, preservatives, solubilizers, emulsifiers and adjuvants. Other embodiments of the compositions of the invention incorporate particulate forms, protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including but not limited to intravenous, intramuscular, parenteral, pulmonary, nasal and oral.

As used herein, an "effective amount" is the amount required to achieve a clinically significant effect. For example a significant reduction of infection, or

reduction of cell growth or reduction of tumor size is a reduction of preferably of at least 30 percent, more preferably of at least 50 percent, most preferably of at least 90 percent. Accordingly, the effective amount will vary with the subject being treated, as well as the condition to be treated. For the purposes of this invention, the methods of administration are to include, but are not limited to administration cutaneously, subcutaneously, intravenously, parenterally, orally, topically, or by aerosol.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a polypeptide analog or fragment of the provided peptide or peptide composition, a peptidomimetic composition thereof as described herein as an active ingredient. A cocktail of the provided pharmaceutical composition in various combinations is also contemplated.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium,

calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

As used herein, the term "synthetic amino acid" means an amino acid which is chemically synthesized and is not one of the 20 amino acids naturally occurring in nature. As used herein, the term "biosynthetic amino acid" means an amino acid found in nature other than the 20 amino acids commonly described and understood in the art as "natural amino acids."

As used herein, amino acid residues are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a

polypeptide. Abbreviations for amino acid residues are used in keeping with standard polypeptide nomenclature delineated in *J. Biol. Chem.*, 243:3552-59 (1969).

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues.

Amino acids with nonpolar R groups include: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan and Methionine. Amino acids with uncharged polar R groups include: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine and Glutamine. Amino acids with charged polar R groups (negatively charged at Ph 6.0) include: Aspartic acid and Glutamic acid. Basic amino acids (positively charged at pH 6.0) include: Lysine, Arginine and Histidine (at pH 6.0). Amino acids with phenyl groups include: Phenylalanine, Tryptophan and Tyrosine. Particularly preferred substitutions are: Lys for Arg and vice versa such that a positive charge may be maintained; Glu for Asp and vice versa such that a negative charge may be maintained; Ser for Thr such that a free -OH can be maintained; and Gln for Asn such that a free NH_2 can be maintained. Amino acids can be in the "D" or "L" configuration. Use of peptidomimetics may involve the incorporation of a non-amino acid residue with non-amide linkages at a given position.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces -turns in the protein's structure.

As used herein, "pM" means picomolar, "nM" means nanmolar, "uM, means micromolar, "mM" means millimolar, "ul" or "µl" mean microliter, "ml" means milliliter, "l" means liter.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however,

as limiting the broad scope of the invention. While the invention is described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

EXAMPLES

EXAMPLE 1: Molecules specifically expressed in murine stem/progenitor cells

Protein tyrosine kinases and phosphatases expressed in murine hematopoietic stem cells have been previously identified (Matthews, W., et al. 1991; Matthews, W., et al. 1991; Dosil, M., et al. 1996). These molecules play important roles in hematopoiesis and development (Dosil, M., et al. 1996; Mackarehtschian, K., et al. 1995; Shalaby, F., et al. 1995; Kabrun, N. , et al. 1997). The present invention contemplates an even more global approach in order to identify molecules specifically expressed in the murine stem/progenitor cell hierarchy. For most of these studies purified (AA4.1+Lin-/loSca+ckit+) fetal liver cells were used. This population is approximately 1,000-fold enriched for in vivo repopulating activity (LTRA) measured by competitive repopulation (Ly5.1/Ly5.2 congenic system) (Moore, K.A., et al. 1997) . All myeloid and lymphoid lineages in primary and in secondary recipients are repopulated by these cells. This degree of enrichment is comparable to the current "state of the art." Other primitive members of the stem/progenitor cell hierarchy share this same cell surface phenotype. These include: (1) LTCIC or cobblestone area forming cells (Ploemacher, R.E., et al. 1989; Ploemacher, R.E., et al. 1991) , (2) CFU-blast progenitors , (3) HPP-CFC progenitors (Lowry, P.A., et al. 1995) and (4) stromal-dependent B-lymphoid progenitors (Whitlock, C.A., and Müller-Sieburg, C.E. 1990) . The AA4.1+Lin-/loSca-ckit+ subset is depleted of LTRA but contains significant in vitro progenitor activity. In contrast, no stem/progenitor cell activity is found in the AA4.1 subset (Jordan, C.T., et al. 1990) . Short-term (5-7 days) cytokine cultures of stem cells were used to generate committed progenitor populations at the expense of LTRA (Traycoff, C.M., et al. 1996; Peters, S.O., et al. 1995; Knobel, K.M., et al. 1994; Yonemura, Y., et al. 1996). In summary, several

cell populations were defined which represent the beginning, the middle and the end points of the hematopoietic hierarchy. This sets the stage for a comparative analysis of gene expression patterns. A goal of the present invention is to complement the physical and functional phenotypes of stem/progenitor cells with profiles of uniquely expressed genes. It was hypothesized that some of these gene products contribute to the unique biological properties of primitive stem/progenitor cells and therefore are regulators of self-renewal, proliferation, commitment and other processes.

There are a number of ways to compare gene expression profiles which are available to one of skill in the art. It is possible to do this by exhaustive sequencing of representative cDNA collections obtained from stem cell and mature cell sources followed by "electronic subtraction". This approach has several drawbacks. Most importantly, the number of sequences which must be obtained is prohibitive. For a homogeneously pure population this number is on the order of 50,000 (based on approximately 10-20,000 expressed genes in an average cell type and a statistical calculation). In practice, even the most purified stem cell population is heterogeneous. Stem cell enrichment values are only meaningful in relation to an unenriched standard and cannot be converted into an absolute stem cell number. It has been documented that cell populations with the same cell-surface phenotypes can differ in biological activity . The unique properties of stem cells also suggest caution when extrapolating from expressed gene numbers in other cells. In short, the extent of sequencing necessary to ensure complete coverage of gene expression in stem cells is not possible to estimate. Normalization procedures (Uchida, N., et al. 1995; Patanjali, S.R., et al. 1991; Soares, M.B., et al. 1994) designed to "equalize" the mRNA abundance classes are not advisable because they obliterate potentially important quantitative expression differences. Additionally, a high-throughput sequencing effort is not applicable to numerous libraries. Comparisons of gene expression in diverse sources of stem cells will provide valuable information. An elegant technique, Serial Analysis of Gene Expression (SAGE) permits the rapid acquisition of thousands of DNA sequence "tags" (Zhang, L., et al. 1997) . This technology was not considered herein because the size of each sequence "tag" is very small (10 base pair, bp). Therefore, SAGE is only informative in two extremes; exact nucleotide matches or no matches to sequences in the databases. This limits database comparison to the same species from which the "tags" originate. A key component of the strategy presented herein relies on broad bioinformatic database comparisons. In

addition, even with a specific "tag" one still needs to obtain a full-length cDNA clone for functional studies. Analysis of gene expression can be done in single progenitor cells after these form a colony "start" (Brady, G., et al. 1990; Brady, G., and Iscove, N.N. 1993). Replating of the sibling cells in a "start" colony allows the approximation of the lineage potential present in the starting cell. This technique suffers from several drawbacks. First, it is limited to 3', non-coding ends, thus preventing protein database comparisons. Second, the technique relies on cell growth, thus it is not suitable for analysis of quiescent cells. Moreover, approaches to allow colony-formation by true stem cells with a retention of primitive properties are in their infancy (Ball, T.C., et al. 1995; Trevisan, M., et al. 1996). Third, the technique does not take into account stochastic models of stem cell behavior (Ogawa, M. 1993). To accurately reveal physical and functional properties of stem cells it is wise to analyze populations where stochastic differences would average out. Single-cell derived cDNA populations could however, provide valuable "adjunct" material for more refined gene expression screens. There are many techniques to physically identify differentially expressed genes. For these and other reasons, most notably the technical and economic ease with which physical pre-enrichments of cDNA libraries can be achieved, the studies described herein began with cDNA libraries which are highly enriched in differentially expressed sequences. The integration of individual techniques served to overcome the inherent limitations of each technique. Three strategies were successfully employed: (1) Differential Display (DD) (Liang, P., et al. 1994; Bauer, D., et al. 1993), (2) Representational Difference Analysis (RDA) (Braun, B.S., et al. 1995; Hubank, M., and Schatz, D.G. 1994; Diatchenko, L., et al. 1996) and (3) standard subtractive hybridization (Li, W.-B., et al. 1994; Harrison, S.M., et al. 1995) . The latter underlies the present invention. A key feature is that the differentially expressed cDNAs have a high probability of being full-length. This facilitates a rapid transition to functional studies. The two former techniques were utilized because of "visual" nature (DD) and the ability to generate representative, differentially expressed probe populations (RDA) in a rapid manner.

Murine stem cell gene expression profiles. As a first step, a series of high quality, representative cDNA libraries were generated. The cDNA populations were directionally cloned into the pSport-1 or pSport-2 plasmids (BRL-Gibco). The most important libraries originate from purified stem cells. In one case, enough AA4.1+Lin-/loSca+ckit+cells were purified to allow construction of a non-based library using

standard methodologies. This library contains ~4x106 independent recombinants (average cDNA insert size of 1-2 kilobases, kb). A second library was constructed using a new PCR-based technology called cap-finder (Clontech) designed to yield full-length cDNA copies. cDNAs ranging from 1 to 4 kb were commonly attained using this technique. For cap-finder procedures the purified cells were processed into DNAse-I digested, poly-A+ mRNA according to microscale procedures routinely in use. The synthesis of cDNA was done with an aliquot of mRNA corresponding to approximately 20,000 cell equivalents. It has not been necessary to use less material, therefore this is not a lower limit. A Not I restriction site was included in the 3' reverse transcriptase primer (cap-finder, version 2) to facilitate directional cloning. An aliquot of the cDNA was amplified for varying PCR cycle numbers, and analyzed by Southern blots (pseudo-Northerns) for the presence of full-length copies of b-actin, GAPDH, CD18, flk2/flt3, cdk4, CD34 and other mRNAs. Optimal cycle numbers were used to amplify the remaining cDNA. The cDNA was cloned into the pSport-1 plasmid. The AA4.1+Lin-/loSca+ckit+ cap-finder library contains ~3 x106 independent recombinants. Single-pass sequence analysis of random clones from this library indicated that 95% were full-length (based on sequences with an exact match in Genbank). Much more extensive sequencing of numerous clones from subtracted libraries has confirmed this. Other libraries constructed in similar ways include two libraries from AA4.1 cells, and two libraries from AA4.1+Lin-/loSca-ckit+ cells. Finally, a library was constructed from AA4.1+Lin-/loSca+ckit+ cells cultured for 5-7 days in a differentiation promoting cytokine cocktail (IL3, IL6, KL). Competitive repopulation and in vitro progenitor assays confirmed a complete loss of LTRA with a significant retention of progenitor cells. All of the cDNA libraries are large (> 2x106 independent recombinants) and of high quality (1-2 kb average insert size). In summary, the most primitive, intermediate and most mature members of the hematopoietic hierarchy were "converted" into representative panels of expressed genes.

Subtractive Hybridization. These cDNA libraries were used in subtractive hybridizations to enrich for differentially expressed genes. Target libraries from AA4.1+Lin-/loSca+ckit+ cells were subtracted with an AA4.1 driver cDNA library. This yields a population of cDNA clones which is enriched in sequences expressed in primitive stem/progenitor cells but not in mature cell types. In practice, a single-stranded target library is hybridized to an excess of in vitro synthesized biotinylated RNA from the

driver library as described . The opposite orientations of the cloning sites in pSport-1 and 2 insure target and driver complementarity. The target library was enzymatically converted to a single-stranded form using gene II protein and exonuclease III (Gene-Trapper protocols, BRL-Gibco). Previously, this was done by infection with M13 helper phage often resulting in a bias for small cDNA inserts. Here, driver/target combinations were subtracted two times in order to facilitate the removal of commonly expressed sequences (verified by elimination of "housekeeping" genes such as b-actin and GAPDH). Concomitant enrichment of known, differentially expressed genes is also verified. Generally flk2/flt3 and CD34 probes were used. Both are expressed in the AA4.1+Lin-/loSca+ckit+ subpopulation but not in the AA4.1 population. Following subtraction, the relative number of clones is reduced by up to 200-fold. In some cases (subtractions with AA4.1 material) the number of clones which "survive" the subtraction is on the order of 10-20,000. Because individual sequences may be represented more than one time, this does not necessarily imply that there are 10-20,000 differentially expressed genes. The exact number of unique sequences (complexity) in the pool of subtracted clones must be determined. A more thorough discussion of complexity is found in a subsequent section. These subtracted libraries should be enriched for sequences expressed in the primitive portion of the stem/progenitor cell hierarchy; that is in stem cells and/or in primitive clonogenic progenitor cells. Two other subtracted libraries, potentially enriched for sequences expressed in the most primitive stem cell but not in clonogenic progenitors were derived by subtracting the AA4.1+Lin-/loSca+ckit+ libraries with material from the closely related AA4.1+Lin-/loSca-ckit+ subpopulation.

Each subtracted library is arrayed at high density onto nylon membranes. Each clone in the array has a unique address in microtiter plates. A density of 20-30,000 clones on a 22 x 22 cm. membrane is practical. Because of the inherent "noise" in any subtraction scheme, a positive selection criterion may be imposed in order to focus on true differentially expressed sequences. Details are presented in a subsequent section. To analyze the subtracted libraries, a high throughput sequencing effort was employed using three libraries. These are: 1) two AA4.1+Lin-/loSca+ckit+ cell libraries (standard and cap-finder) subtracted extensively with AA4.1 cell material and 2) a standard AA4.1+Lin-/loSca+ckit+ library subtracted with AA4.1+Lin-/loSca-ckit+ material. An average of 400 bp of 5' sequence was obtained from about 1000 clones. To facilitate a rational handling of sequence information and to focus attention on a small number of

clones for functional analysis a "flow of information" strategy was devised. (See, Figure 1).

A major component of this strategy is bioinformatics. This can be global (comparisons with outside databases) and local ("in-house" analyses within an individual library or comparison of several libraries). A relevant example of the latter is comparison of genes from murine stem cells with genes derived from their human counterparts. Global bioinformatic analysis provides much information. First, it establishes if a given sequence corresponds identically or closely to an already identified gene in the mouse, human or other mammalian species. Such homologies can be detected at the nucleotide level. This can provide evidence of hematopoietic expression for a previously described gene. Second, where the homologies are statistically significant but not identical, novel members of gene/protein families can be identified. Third, a wider net can be cast over the databases by using conceptual translation of a sequence in the homology comparisons. The Examples provided herein illustrate the power of this approach in revealing similarities to proteins from invertebrate organisms such as Drosophila, C. elegans, and even yeast. In many cases these proteins have functions which have been uncovered by the analysis of mutants. Based on protein homologies "virtual links" are drawn between developmental regulation in invertebrates (such as in germ-line development) and in hematopoietic stem cells. The Notch/Notch ligand pathway first defined in invertebrate cell-fate determination and recently implicated in hematopoietic regulation is a good example .

A number of other putative proteins which share homology with key Drosophila proteins have been identified. Bioinformatics facilitates the recognition of peptide motifs such as EGF-like repeats, Ig-like domains or Zn-finger modules. Fourth, because the databases are annotated, predicted protein sequences can be assigned to cellular processes such as signal transduction pathways or apoptosis. It is also possible to categorize clones according to potential involvement in other mammalian stem cell systems such as the intestine. Fifth, it is feasible to perform virtual expression studies and to construct overlapping EST contigs which can yield virtual full-length cDNAs.

The following discussion summarizes the general murine findings and highlights a panel of "interesting" subtracted clones. A number of full-length sequences have been determined. Bioinformatic analysis summaries on a collection of 863 clones derived

from AA4.1+Lin-/loSca+ckit+ libraries subtracted with AA4.1 RNA are shown in Table 1.

The present invention encompasses numerous bioinformatic search and comparison parameters. This is the first analysis of its kind in the hematopoietic system and several important points emerge. First there is a high proportion (~50%) of novel sequences. Sixteen percent do not show homologies in any EST database queried. A preliminary analysis of a small sequence set from an AA4.1+Lin-/loSca+ckit+ library depleted of sequences in common with the AA4.1+Lin-/loSca-ckit+subset has indicated an even higher percentage of novel genes. These are higher percentages than would emerge from a random analysis of an unsubtracted library. This was directly addressed by performing these analyses on a similar number of sequences selected at random from one of the mouse EST databases. Second, among the ~50% of the clones which are significantly homologous to previously identified genes or proteins, the proportion of homologies to "housekeeping" genes is low. This underscores the effectiveness of the subtraction strategy. Third, an internal analysis of the data base has revealed few redundancies. This illustrates the degree of gene expression diversity between the two endpoints of the mouse hematopoietic hierarchy; thus supporting the starting hypothesis. A recent report has suggested that many sequences in mature blood cells are expressed in primitive, non-committed cells (Hu, M., et al. 1997). The data indicate that many of these are removed by the subtraction; thus uncovering a large, previously not described set of genes. Fourth the sequences with homologies to known genes or proteins can be subdivided according to protein families and putative function. Interestingly, a large percentage (32%) fall into the signaling protein category. Examples of these are described herein below.

As part of a more sophisticated bioinformatics approach, automation of database searches, information cross-referencing, and annotation was employed. An illustrative example is automated weekly database queries with the sequence set. New, previously-unidentified homologies were automatically noted and reported. In the mouse studies several genes were encountered whose expression would be predicted to differ between the AA4.1+Lin-/loSca+ckit+ and the AA4.1- populations. Both murine CD34 and flk2/flt3 were identified in the sequenced population. This provides a good internal control for the screening strategy. As shown in Table 2, a "short list" of identified

molecules was generated based on bioinformatics and in many cases, expression-specificity verification. The features of some of the molecules merit discussion.

The SA49P1 clone is homologous to sex comb on midleg; a member of the Drosophila Polycomb group of zinc-finger transcriptional repressors (Bornemann, D., et al. 1996). Polycomb proteins are regulators of homeobox (HOX) genes and maintain the developmental stability of transcriptional states (Simon, J. 1995) . There is currently great interest in HOX gene function in the biology of hematopoietic stem cells (Sauvageau, G., et al. 1995; Lawrence, H.J., et al. 1996) . The C4-80 gene is homologous to the Drosophila cornichon gene (Roth, S., et al. 1995) . Cornichon is required during oogenesis for the induction of follicle cells, which provide the environment that supports oocyte development. Cornichon is a component of the Drosophila EGFR signaling pathway (Neuman-Silverberg, F.S., and T. Schupback 1996). The LL2-12 gene is similar to the Drosophila neurogenesis gene brainiac. Mutants defective in this extracellular gene product show neural hyperplasia. Brainiac also plays a role in oogenesis (Goode, S., et al. 1992; Goode, S., et al. 1996) . Mosaic experiments show that brainiac is required in the developing oocyte. Brainiac involvement in the Drosophila EGFR pathway has been suggested. The LL2-35 gene is similar to the Drosophila germ cell-less gene (Jongens, T.A., et al. 1992) . The product of this gene is required for the specification of the Drosophila germ line. The ectopic expression of germ cell-less causes somatic cells to adopt the characteristics of pole cells (destined for the germ line). The homologies of the above clones to Drosophila genes were identified at the predicted amino acid level. This underscores the utility of the bioinformatic approach. In all four cases the cDNAs represent novel unpublished murine genes. Clearly, the involvement of three of the Drosophila genes in the ultimate stem cell system, the germline, coupled with the identification of homologous genes expressed in mouse hematopoietic stem /progenitor cells gives food for thought. Four other cDNAs identified are homologous to the Drosophila genes dishevelled and smoothened (mouse homologs are already described) as well as to kelch and discs large (not previously described in the mouse). DD116 was originally isolated in a DD comparison of AA4.1+ vs. AA4.1 cells. A cDNA clone identified with the DD116 probe was sequenced; the predicted protein is homologous but not identical to the murine beige gene product. The beige mutation in the mouse causes bleeding, immune system disorders and a coat-color phenotype (Perou, C.M., et al. 1996; Fukai, K., et al. 1996; Barbosa, M.D., et al. 1996). Murine beige is thought to be the

homologue of a gene responsible for the Chediak-Higashi syndrome in human. This novel gene is likely to be the second member in the beige family; it is designated herein as "taupe". Two of the cDNAs in Table 2 were identified in a DD comparison of AA4.1+Lin-/loSca+ckit+ cells and cytokine differentiated, cultured cells. One focus was molecules whose expression disappeared with the loss of stem cell activity in culture. Cyt28 and Cyt19 fulfilled this criterion and were used to isolate full length clones which have been completely sequenced. Cyt28 encodes a novel, seven-transmembrane domain receptor in the same family as mouse F4/80 and human CD97 (secretin receptor superfamily) (Hamann, J., et al. 1995; Baud, W., et al. 1995) . Cyt 19 is a novel putative methyltransferase. In both of these cases protein family assignations were made after extensive bioinformatic analyses. At least in the case of Cyt28, an antibody will be a useful reagent because it may provide a means to further subdivide the stem/progenitor cell population. Such antibodies are provided by the present invention. The cDNA SA61 is similar to a newly discovered molecule called p62dok. p62dok is a tyrosine phosphorylated protein which binds to rasGAP and is likely to be a common target for numerous tyrosine kinases including Abl and ckit (Carpino, N., et al. 1997; Yamanashi, Y., 1997) . Interestingly, p62dok is also constitutively phosphorylated in hematopoietic progenitor cells from chronic phase CML patients (Carpino, N., et al. 1997) . The predicted protein encoded by SA61 appears to be a second member of this protein class. Other representative molecules are listed in Table 2. Two cDNAs were identified as homologous to putative apoptosis regulators (SBSA56 and LL5-68, two cDNAs homologous to genes translocated in leukemias (LL5-03 and B2-67), several homologies to putative chromatin proteins (C4-23, C3-25 and LL2-89) and a LIM-domain encoding cDNA (LL5-96). Most interestingly, three cDNAs (B3-77, C2-48, and LL2-76) are homologous to genes expressed in intestinal crypts or during intestinal development.

Isolated cDNAs were hybridized to slot blots representing globally amplified cDNAs from pools of individual progenitor and mature cells. Because the developmental potentials of these cells have been measured (by replating of siblings) it is possible to graphically represent the expression of a gene in various stages of the hierarchy. Three examples are shown in Figures 2A-2C. The Smc-34 cDNA is a completely novel sequence with a predicted leucine zipper and several potential membrane spanning domains. These examples underscore the value of "interfacing" the above-described approach with a single cell approach. Thus, it was demonstrated that a given gene is

expressed in at least multipotential progenitor cells and other members of the progenitor cell hierarchy.

Human stem cell gene expression profiles. It is also a goal of the present invention to identify genes expressed specifically in human stem cells. It is useful to use a multi-species (mouse and human) approach to define stem/progenitor cell-specific gene profiles. One goal is to integrate mouse and human information. However, rather than proceeding directly to human homologs of the mouse genes already identified, an independent human effort was initiated. The rationale for this is multi-faceted. First, it is possible that some aspects of human and mouse stem cell biology are regulated in different ways. Although it is likely that most regulatory pathways will be conserved, it must be kept in mind that many properties ascribed to the most primitive stem cell population have been rigorously proven only in the mouse. Clearly a human lymphoid-myeloid stem/progenitor cell exists. However, the exact degree of proliferative capacity (the ability to give rise to oligoclonal hematopoiesis) as well as the exact spectrum of differentiation potentials of human stem cells have not yet been accurately measured. Several potential differences between mouse and human have already been mentioned. One additional indicator of differences may be the difficulty of gene-transfer into stem cells from large animals (Larochelle, A., et al. 1997). This is not likely to result solely from the quiescent status of the most primitive cells. Second, while it is usually possible to find human homologs for individual mouse genes by manipulating hybridization stringency, such conditions will vary for different genes. Therefore, to find human homologs for a large pool of mouse genes (i.e. 100) may be more labor intensive and costly than to independently determine a sequence profile of differentially expressed genes from human stem/progenitor cells. Relationships to the murine panel can then be determined electronically where it is easy to manipulate comparative parameters. Clearly, for some individual mouse genes it will be of great importance to physically identify human homologs. In some cases it may be possible to use existing human EST databases to quickly obtain the sequence of a human homolog. Third, the availability of a large panel of human sequences specifically expressed in stem/progenitor cells lends itself to the application of various chip and array technologies. Such technologies will be instrumental in identifying which subsets of human stem cell specific genes are up or down-regulated in the highly clonogenic stem cells from diseases such as Acute Myelogenous Leukemia (AML) and other leukemias.- Highly purified human stem

cells for cDNA libraries were obtained from normal donor BM. CD34+ cells were obtained from the mononuclear fraction using an immuno-magnetic affinity device (Miltenyi MACS column) and stained with a mixture of monoclonal antibodies (mAbs) designed to identify both primitive and mature cell surface markers. To identify antigens on mature cells a lineage (Lin) cocktail of FITC conjugated mAbs was used . These mAbs were directed against: CD3, CD11b, CD15, CD19, CD20, glycophorin A, and CD71 (CD3, CD15, CD19 and CD20 from Becton Dickinson, CD11b and glycophorin A from Coulter, and CD71 from Ortho Diagnostics). To identify primitive cells mAbs were used which were directed against CD34 (biotin conjugated and detected via a streptavidin-allophycocyanin reagent; Coulter) and either CD38 (phycoerythrin conjugated, Becton Dickinson) or CD90 (Thy1) (phycoerythrin conjugated; Pharmingen). The stained cells were analyzed using a dual laser Becton Dickinson FACStar Plus flow cytometer. Cells of the desired phenotype were sorted into siliconized tubes. To obtain material from cultured cells, CD34+ enriched populations (isolated as described above), were first cultured in serum-free medium (IMDM, 5 mg/ml low density lipoprotein, 2 mM glutamine, human serum albumin, insulin, and transferrin,) supplemented with IL-3 (5ng/ml), KL (25ng/ml), and FL (25ng/ml). After 1-4 days of culture, the cells were purified as described above. Human libraries were constructed using the cap-finder technology, version 2. Amplified cDNAs originating from several independent BM donors were pooled. Representative cDNA libraries have been constructed from the following sources: (1) BM CD34+Lin- cells (7.1X105 independent recombinants), (2) BM CD34+Lin-CD38+ cells (1.9X106 independent recombinants), (3) BM CD34- cells (1.6X106 independent recombinants), (4) CB CD34+ cells (4.3X105 independent recombinants), and (5) CB CD34- cells (2.9X105 independent recombinants). The average cDNA insert size in all libraries is 1-2 kb. According to the comparative biological properties of the source material used for the mouse and human cDNA libraries, the following parallels can be drawn: (1) mouse AA4.1+Lin-/loSca+ckit+ ≅ human CD34+Lin-, (2) mouse AA4.1+Lin-/loSca-ckit+ ≅ human CD34+Lin-CD38+ and (3) mouse AA4.1 ≅ human CD34-. Thus, in both species, collections of clones representing the beginning, the middle and the end-points of the hematopoietic hierarchy have been generated. Using procedures described above, the human libraries were subtracted and arrayed 3,000 clones from two libraries: (1) BM CD34+Lin- subtracted

with BM CD34+Lin-CD38+ material (referred to as: 38-)38+) and (2) BM CD34+Lin- subtracted with BM CD34- material (referred to as: 38-). Before initiating a sequencing effort pilot studies were performed to improve the resolution of the screen. Specifically, in order to develop techniques which would eliminate as much non-specific "noise" as possible from the libraries. RDA (PCR-Select, Clontech) were used . There are several salient features of RDA. First, it is rapid (2-3 days) and applicable to small cell numbers (ie. several thousand). It does not require the generation of cloned cDNA libraries therefore, numerous comparisons can be made simultaneously. Second, it yields short cDNA fragments (generated by 4-cutter restriction enzymes) which are representative of differentially expressed mRNAs. Third, it is based on the suppression PCR technique which prevents overamplification of abundant messages (Diatchenko, L., et al. 1996) . In addition, sequences expressed in both populations in an RDA comparison do not amplify exponentially. Differentially expressed populations of cDNA fragments are not obtained by physical enrichment but rather by selective PCR amplification. Fourth and most importantly, it simultaneously yields two populations each representing differentially expressed genes in one of the two starting samples. This bi-directionality is valuable because as discussed below it can simultaneously reveal up and down regulated genes. RDA comparisons were made between CD34+Lin- and CD34+Lin-CD38+ and between CD34+Lin- and CD34- cDNAs. The latter comparison was verified by hybridization. As shown in Figure 3A (right panel), the control, non-subtracted RDA cDNA population (38-) contains b-actin sequences which are missing in the two subtracted RDA populations, 38- and 38-)38+. A differentially expressed gene (HDD-2, described below) is enriched in the 38- RDA population and at least retained in the 38-)38+ RDA population (Figure 3A, left panel). Two, bi-directional, RDA cDNA populations (38- and the converse 34-)38) were used to probe (See Figure 3) duplicate arrays of a subtracted 38- library (Figure 3B). The correct RDA probe hybridizes to considerably more clones than the incorrect probe (compare the greater numbers of spots in the left as compared to the right panel). Hybridization signals for each clone are doublets due to the arraying technique. Hybridization signals with the incorrect probe (right panel) suggest that further improvements to this strategy are worth pursuing. Because the subtractions are based on different technologies, it was reasoned that clones which "survived" the library subtraction and hybridized preferentially to the "correct" RDA probe would be more likely to represent true differentially expressed genes. Preliminary data from the mouse

studies had already suggested that this was indeed the case. The arrayed clones were also hybridized to probe populations from the entire starting non-subtracted libraries in order to eliminate residual cDNA clones corresponding to abundant mRNA species.

In one analysis, a total of 77 clones (49 from the 38- subtraction and 28 from the -38-)38+ subtraction) which "obeyed" the RDA criteria were sequenced from the 5′ end. In order to verify expression specificity, a series of pseudo-Northern blot analyses were performed. cDNA populations from independent purified sources of material was amplified. This is of particular importance with an outbred species such as humans. In these experiments additional material from CD34+Lin-CD90+ (Thy1+) (three separate purifications) and their CD90- (Thy1-) counterpart subpopulations was also included. Cytokine-cultured samples were also included. These were CD34+Lin- cells and their CD38+ counterparts purified after 1, 2 and 4 days of culture. The collective panel of cDNAs also includes four independently purified CD34+Lin- populations and their CD34+Lin-CD38+ counterparts. All of these amplified cDNAs have been arranged on numerous pseudo-Northern blots. In short, this allowed us to evaluate the expression of a given "sequence tag" in stem cell populations isolated by two different criteria, from a number of independent donors and after cytokine culture. Several interesting genes emerged from the sequence data set. Individual clones obtained from the -38-)38+ subtraction are designated 38 letter/number (i.e., 38A1) while those from the 38-)34+ subtraction are designated 34 letter/number (i.e., 34A1). One clone (38B5) was identified and determined to be human flk2/flt3. A second interesting clone (34B4) is closely related to a gene encoding TINUR (Figure 4A). Clone 34B4 may be a novel variant of TINUR due to a 25 amino acid, in-frame deletion. TINUR was identified as an orphan member of the steroid receptor superfamily (NGFI-B/nur77 subfamily)(Okabe, T., et al. 1995). TINUR has also been implicated in apoptosis. An additional clone (34F4) is highly homologous to DAP-kinase. This protein is a serine-threonine kinase which has been implicated in cytokine (IFN- -induced apoptosis (Deiss, L., et al. 1995) . Clone 34F4 (DAP-Kinase) and 34B4 (TINUR) both exhibit a stem-cell restricted expression pattern. Clearly, the identification of two genes whose products are implicated in apoptosis and whose expression is largely restricted to human stem cells is of interest. In addition, a cDNA (34A5) was identified which is closely related to the MLF1 gene which is a translocation partner in t(3;5)(q25.1;q34)(Yoneda-Kato, N., et al. 1996) . This translocation is associated with Myelodysplastic Syndrome (a stem cell disease) and

AML. The sequence homologies and restricted expression pattern of 34A5 is shown in Figures 4B and 4C. In Figures 4C and 4D (and also 5A, 5B, and 6B) there are twenty-one samples of capfinder-amplified cDNA from various hematopoietic populations. From left to right these are: four CD34+Lin- populations, three CD34+Lin-CD90+ populations, two CD34- populations, four CD34+Lin-CD38+ (obtained from the same BMs as the CD38- samples in lanes 1-4), two CD34+Lin- samples (obtained from the same BMs as the CD90+ samples in lanes 5 and 6), three CD34+Lin- populations obtained after 1, 2 or 4 days of culture and finally their three CD34+Lin-CD38+ counterparts. A recent study shows that, at least with some types of AML, the disease can be transferred into NODSCID mice only by the leukemic CD34+CD38- subpopulation (Bonnet, D., and Dick, J.E. 1997) . These demonstrate MLF1 expression in normal stem cells. As shown in Figure 4D, nucleophosmin (NPM), the partner in this translocation was also identified. This is an example of non-specifically expressed "noise" in the screens. The 38G2 cDNA is closely homologous to the LTG9/MLLT3 gene located on 9q22 and involved in t(9;11) leukemia (Iida, S., et al. 1993) (Figure 4E). A degree of stem cell expression specificity has also been observed. All of the above cDNA clones are likely to be identical to the homologous, previously identified genes. However, the suggested involvement of DAP-kinase and TINUR in apoptosis necessitates their inclusion in any comprehensive consideration of stem cell apoptotic pathways. Similarly, the expression of two genes associated with myeloid leukemias bears on any speculation regarding the primary transformation target cell as well as the origins of the ultimate clinical phenotype in these and other leukemic disorders.

Other known genes to were found that exhibit identity or very close homology including: (1) G0S3, a fos-related gene (Heximer, S., et al. 1996) (Figure 5A) and (2) HLA-DR (Figure 5B). G0S3 shows a specific expression pattern, while HLA-DR expression appears to be more variable. The expression status of Class II MHC on the most primitive human BM stem cell population is not entirely clear. It has been suggested to be present in a primitive, multipotent progenitor population but not in the most primitive stem cells (Sutherland, H.S., et al. 1989; Verfaillie, C., et al. 1990) . If true, this may suggest an additional negative selection parameter for future experiments designed to subdivide the stem/progenitor cell hierarchy. Of the first 75 sequences, 22 have no homologies in the databases or homologies only to ESTs. Expression analyses on these clones are in progress. One gene of particular interest is called HDD-2 as well as

34B5, 34E1 and 38A11. The three latter designations reflect its isolation from both the 38- as well as the 38-)38+ subtracted libraries. The designation HDD-2 reflects its independent isolation in a limited DD "first look" at molecular differences in the purified cell populations. The likely full-length sequence of HDD-2 is ~500 bp. It contains a short open reading frame of 89 amino acids (SEQ.ID.No.: 71). The predicted peptide sequence is shown in Figure 6A. The 3' cDNA sequence contains a poly-A tail preceded by the canonical AATAAA poly-adenylation signal. Neither the nucleotide nor the predicted protein sequences of HDD-2 show homologies in any known gene or EST database. The expression profile of HDD-2 shown in Figure 6B, demonstrates that it is stem cell restricted. Also shown below (Figure 6C) is HDD-2 hybridization to a dot blot with numerous human pA+ mRNA samples (Clontech). HDD-2 hybridization is only visible in kidney (the other "spots" are background). It was confirmed that HDD-2 corresponds to a single-copy human gene by genomic Southern blot (Figure 6D). In summary, the results of this very low throughput human sequencing effort substantiate the overall approach; that judicious pre-enrichments and selections will result in rapid identification of biologically interesting and often novel genes. Most importantly, these studies firmly establish the existence of genes whose expression correlates with the most primitive stem cell phenotype.--

Other data were generated using (1) a murine stromal cell line to support enriched human stem/progenitor cells, and (2) tetracycline regulatable retroviral expression vectors. The stromal cell line AFT024 is efficient in long-term, in vitro maintenance of LTRA in purified murine fetal liver and adult bone marrow populations (Terstappen, L.W.M.M., et al. 1991) . Additional data demonstrated highly-efficient retroviral-mediated gene-transfer into murine LTRA as well as into primitive in vitro progenitors during the AFT024 cocultures. Further, it has been shown that AFT024 is very effective in supporting ELTCIC. Specifically, the CD34+CD38- immunophenotype as well as the functional capacity of these cells is maintained. The latter was measured in limiting-dilution replating experiments. Moreover, it has been shown that limiting numbers of human CD34+Lin- cells can give rise to both B cells and NK-cells when cultured on AFT024. However, the supportive activities of AFT024 on mouse and human stem/progenitor cells have been indistinguishable. In addition, more than 500 sequences have been analyzed from an AFT024-specific subtracted cDNA library. A number of candidate stem cell regulators have been identified. Three of these are dlk (Moore, K.A.,

et al. 1997), a novel BMP/TGF-b superfamily member and a novel selectin-related molecule. The present invention contemplates identifying human stem/progenitor cell receptors and/or ligands for the AFT024 specific proteins.

In order to facilitate functional studies of stem cell gene products several retroviral gene-transfer vector systems were constructed and characterized. All of these employ the 293T cell retroviral packaging system (Kinsella, T., and Nolan, G. 1996). High titers of virus can be produced transiently without the time and labor consuming effort required for stable producer cell lines. Large cDNA populations can also be converted into virus populations (Kitamura, T., et al. 1995). It is preferable to have inducible (or repressible) vectors which are also selectable. Also, the single-transcription unit tetracycline (tet) repressible vector was modified (Hofmann, A., et al. 1996). This vector includes an enhancer/promoter deletion in the 3' LTR. The tet system is currently one of the best inducible expression systems available; regulation over a several hundred-fold range of expression is observed (Shokett, P.E., and Schatz, D.G. 1996) . Cloning sites were introduced in order to insert cDNAs in a sense or antisense orientation. A green fluorescent protein (GFP) marker was also included in these vectors driven by a thymidine-kinase promoter (TK-GFP) (Yang, T., et al. 1996; Cheng, L., and Kain, S. 1996). The cDNA fragment of interest is under tet regulation while GFP is constitutively expressed in transduced cells. In order to confirm this, NIH3T3 cells are infected with a LacZ virus and GFP+ cells sorted. The GFP+ cells express LacZ in the absence of tet, while LacZ expression is undetectable in most cells after the addition of tet. The titer of this model construct is approximately 104/ml. which is suitable for tissue culture studies.

Table 1. Known genes (or extensive aa homology): 436 (50.5%)

	Grouped by function	
by nucleotide: 407	Signaling/receptors:	133
by amino acid only: 29	Translational/post-:	62
	Structural:	49
to mouse: 230	Transcriptional/post-:	45
to human/other: 206	Cell fate:	35
	Other:	87
	Unknown function:	25

Novel genes: 427 (49.5%)

Homologous only to expressed sequence tags: 288 (33.3%)
No homology to any known nt or aa sequences: 139 (16.2%)

Table 2.

Putative family	Clone	Method	Cells compared	Notes
7-transmembrane receptor	Cyt-28	diff. display	SC* fresh vs. 7d-cultured	secretin R superfamily
Methyltransferase	Cyt-19	diff. display	SC fresh vs. 7d-cultured	
	C3-54	subtraction	SC vs. AA4$^-$	
Aspartyl protease	SA7	subtraction	SC vs. AA4$^-$	
Signal transduction molecules	SA61	subtraction	SC vs. AA4$^-$	dok family member
	LL2-02	subtraction	SC vs. AA4$^-$	BTK associated
beige-related proteins	DD116	diff. display	AA4$^+$ vs. AA4$^-$	contains WD repeat
Transcriptional regulators	SA49P1	subtraction	SC vs. AA4$^-$	polycomb homol.
	LL5-96	subtraction	SC vs. AA4$^-$	includes LIM domain
G-protein signaling	LL4-39	subtraction	SC vs. AA4$^-$	cell cycle transition
	B1-66	subtraction	SC vs. AA4$^-$	similar to BL34
Apoptosis-related genes	SBSA56	subtraction	SC vs. AA4$^-$	SMT3A-related
	LL5-68	subtraction	SC vs. AA4$^-$	sim. to Requiem transcript. factor
Chromatin proteins	C4-23	subtraction	SC vs. AA4$^-$	yeast HST2-like
	C3-25	subtraction	SC vs. AA4$^-$	NHP2-like
	LL2-89	subtraction	SC vs. AA4$^-$	yeast SIS2-like
Other stem cells (intestinal	B3-77	subtraction	SC vs. AA4$^-$	homologous to A4 gene
crypts)	C2-48	subtraction	SC vs. AA4$^-$	homologous to C101
	LL2-76	subtraction	SC vs. AA4$^-$	homologous to EDPF
Genes involved in leukemogenic	LL5-03	subtraction	SC vs. AA4$^-$	t(1;11)(q21;q23)
translocations	B2-67	subtraction	SC vs. AA4$^-$	t(X;14)(q28;q11)
Homologues of Drosophila	LL2-35	subtraction	SC vs. AA4$^-$	germ cell-less
developmental genes	LL2-12	subtraction	SC vs. AA4$^-$	brainiac
	C4-80	subtraction	SC vs. AA4$^-$	cornichon
	B4-14	subtraction	SC vs. AA4$^-$	discs-large

*The designation "SC" refers to sorted day 14 fetal liver cells having the phenotype AA4.1$^+$Lin$^-$Sca$^+$ckit$^+$.

EXAMPLE 2: Molecular cloning and characterization of AA4, an early marker of hematopoietic development

In this example, the expression cloning and molecular characterization of AA4, a surface marker expressed on hematopoietic stem and progenitor cells is described. The results demonstrate that AA4 is a 130kDa type I glycosylated membrane protein whose structural organization suggests a role in cell adhesion. Expression analysis showed that high levels of AA4 are found in lung, heart, and bone marrow. It is not found in undifferentiated ES cells, but its expression is upregulated as these cells differentiate into colonies of hematopoietic precursors and endothelial cells. In the hematopoietic system, expression of AA4 correlates with the expression of stem cell markers CD34 and CD43. Functional studies revealed that AA4 coimmunoprecipitates with CD34 and CD43, suggesting that these proteins form a macromolecular complex which functions in the regulation of cell adhesion, proliferation and/or differentiation of hematopoietic cells.

Introduction

Monoclonal antibody AA4.1 was first described more than a decade ago (McKearn et al., 1984) and since then it has become a useful marker for the isolation and analysis of hematopoietic cells (McKearn et al., 1985; Jordan et al., 1990; Fujimoto et al., 1996). A number of works have shown that the antigen recognized by AA4.1 is present on a subset of primitive hematopoietic progenitors found at various stages of development in sites of active hematopoiesis in yolk sac (Cumano et al., 1993; Auerbach et al., 1996; Yoder et al., 1997), AGM region (Godin et al., 1995; Marcos et al., 1997), fetal liver (McKearn et al., 1985; Jordan et al., 1990; Cumano and Paige, 1993), and bone marrow (Li et al., 1996; Szivassy and Cory, 1993). In yolk sac, AA4-positive cells are first detected at day 8-10 of gestation (Cumano et al., 1993; Sanchez et al., 1996). At day 9-10 of gestation $AA4^+c\text{-}Kit^+$ progenitors are found in the P-Sp/AGM region (Sanchez et al., 1996; Marcos et al., 1997), and by day 14 of development, AA4 defines 0.5-1.0% of the fetal liver tissue that contains the entire hierarchy of primitive hematopoietic cells (Jordan et al., 1990). Proliferation within each successive compartment results in increased total number of progenitor cells. Antigen density per cell also increases with developmental progress, which is especially marked for c-Kit and AA4 (Marcos et al., 1997). In bone marrow, HSC are found in both $AA4^+$ and $AA4^-$ subpopulations, although in adult marrow AA4 is largely regarded to be a marker of early B lymphoid lineage. In addition,

recent studies demonstrate that the expression of AA4 parallels the onset of hematopoietic development in differentiating ES cells (Kabrun et al., 1997; Lin and Neben, 1997; Potocnik et al., 1997). Taken together, these results indicate that AA4 plays an important role in hematopoiesis and has to be studied in more detail.

In order to achieve this goal, AA4 was molecularly cloned and characterized. The results demonstrate that AA4 is a 130kDa type I transmembrane glycoprotein whose structure suggests a role in cell adhesion. Expression analysis showed that high levels of AA4 are found in lung, heart, and bone marrow. In the hematopoietic system, expression of AA4 correlates with the expression of stem cell markers CD34 and CD43. Functional studies indicate that AA4 coimmunoprecipitates with CD34 and CD43, suggesting that these proteins form a macromolecular complex which may function in the regulation of cell adhesion, proliferation and/or differentiation of hematopoietic cells.

Results

Cloning of AA4. In order to identify AA4, AA4.1 monoclonal antibody was used to screen various cell lines and primary hematopoetic cells. The murine B lymphoid cell line D2N was found to expresses relatively high levels of AA4 antigen (see Table 3). Immunoprecipitation of protein extracts prepared from D2N cells showed that AA4.1 recognizes a protein with apparent molecular weight (M_r) 130kDa (Figure 7). This protein was also present in extracts prepared from B lymphoid cell line M2.4, and AA4 positive hematopoietic cells derived from bone marrow and fetal liver.

To isolate AA4, a cDNA library was prepared from D2N cells and cloned into the polylinker region of a retroviral expression vector REBNA (see Materials and Methods). Following production of retroviruses, NIH 3T3 cells were infected with the recombinant cDNA library and selected for AA4 expression by flow cytometry using PE-conjugated AA4.1. After two rounds of sorting, genomic DNA extracted from AA4-positive cells was analyzed by pcr amplification using viral vector primers. This resulted in the amplification of a 3.1 kbp cDNA which was gel-purified and subcloned for further analysis. Infection of NIH 3T3 fibroblast or EML C1 hematopoietic cells with REBNA/AA4, a recombinant retrovirus expressing the cloned cDNA, has lead to the acquisition by cells of high affinity to AA4.1 mAb (Figure 8A). In infected cells, AA4.1 detects a 130kDa surface protein which comigrates with the endogenous AA4 from D2N cells (Figure 8B), thus indicating that the cloned cDNA encodes AA4.

Sequence analysis of AA4. Sequence analysis of the cloned cDNA (SEQ. ID No.: 72) showed that it has a single open reading frame encoding a protein of 644 amino acids (see Figure 9)(SEQ. ID No.: 73. The deduced amino acid sequence includes a putative leader peptide and the mature protein which starts at position 20. The protein contains a long N-terminal extracellular region, a single putative hydrophobic transmembrane region, and 47 amino acids of the C-terminal cytoplasmic domain. The extracellular part of AA4 is composed of two major structural moieties. The N-terminal region contains a C-type lectin domain (CTL) which has 32% sequence homology to endothelial cell receptor thrombomodulin. This region is followed by a cysteine-rich domain composed of six epidermal growth factor (EGF)-like repeats, three of which are consistent with the calcium-binding EGF motifs. Similar repeats are found in the extracellular domains of a large number of membrane-bound proteins and in proteins known to be secreated (Bork et al., 1996).

Database searches revealed that AA4 exhibits high homology to C1qRP, the human receptor for complement component C1q expressed on surfaces of myeloid cell lineage and endothelial cells (Nepomuceno et al., 1997). Sequence alignment showed that AA4 and C1qR have approximately 68% identical amino acid positions and similar domain structures. Highest homologies were found within the N-terminal parts of the proteins and their C-terminal cytoplasmic domains (see Figure 10A and 10B), suggesting that AA4 and C1qR may share functional properties.

The amino acid sequence of AA4 contains numerous potential O-linked and N-glycosylation sites. Although the predicted M_r of AA4 is 67.4 kDa, two protein bands exhibiting M_r 105kDa and 130kDa respectively, are immunoprecipitated by AA4.1 mAb in cells infected with REBNA/AA4 (Figure 8C). When ^{35}S-labeled cells are chased with nonradioactive media, the intensity of the 105kDa band rapidly decreases, while the intensity of the 130kDa band increases, thus indicating that p130 is the mature form of the protein, whereas p105 is its precursor. In agreement with this conclusion, immunoprecipitation of biotinyated cells using AA4.1 reproducibly results in the detection of a 130kDa surface protein (see Figure 8B).

Expression patterns of AA4. Northern blot analysis showed that in adult mouse tissues, AA4 is expressed at high levels in lung, heart, and bone marrow. No detectable expression was found in brain, testis, spleen, and thymus (Figure 10B). In normal tissues

and transformed cell lines, a 7kb RNA species hybridizes with the cloned cDNA (Figure 11A and 11B). In addition to the 7kb species, poly(A)-RNA from D2N cells contains a minor band corresponding to a 3.2 kb mRNA (see Figure 11A, lane 8). Similarity search against expressed sequence tags (ESTs) showed that databases contain at least seven different sequences corresponding to the 3'-untranslated region of the cloned *aa4*cDNA which were isolated from the following mouse tissues: colon (Genbank accession No AA929174), heart (AA435107), lymph node (AA185911 and AA267407), lung (AA220480), mammary gland (AI021507), and spleen (AA145088). Sequence identity with the ESTs abrogates upstream of nucleotide G at position 2481 in the 3'-untranslated region of *aa4*cDNA, thus indicating that the cloned cDNA corresponds to an alternatively spliced 3.2 kb *aa4*mRNA.

Expression of AA4 in hematopoietic progenitor cells AA4 is produced in murine hematopoietic progenitor cells and immature B lymphocytes found at various stages of development in yolk sac (Godin et al., 1995; Marcos et al., 1997), fetal liver (McKearn et al., 1985; Jordan et al., 1990), and bone marrow (Cumano et al., 1992; Li et al., 1996). To confirm that the cloned cDNA encodes AA4, RT-PCR was performed on hematopoietic cells fractionationated using several different techniques. The cells analyzed included $AA4^+$ and $AA4^-$ fetal liver (FL) cells; $AA4^+$ FL cells fractionated into $Lin^{lo}c\text{-}Kit^+Sca\text{-}1^+$ and $Lin^{lo}c\text{-}Kit^+Sca\text{-}1^-$ populations; and hematopoietic progenitors isolated from bone marrow (BM) by cell sorting using combinations of different surface markers.

Figure 12A shows that *aa4* was amplified from $AA4^+$ FL cells, whereas in $AA4^-$ cells it was only present at low levels. AA4 expression was high in $Lin^{lo}c\text{-}Kit^+Sca\text{-}1^+$ cells enriched for HSC activity. In adult marrow cells, *aa4* was amplified from Lin^+ cells and $Lin^-c\text{-}Kit^+Sca\text{-}1^+CD34^+$ multipotential progenitors. At lower levels *aa4* was present in $Lin^-c\text{-}Kit^+Sca\text{-}1^+CD34^-$ long term reconstituting HSCs (Figure 12B). In all tested cells *aa4* expression correlated with the expression of stem cell markers CD34 and CD43.

Embryonic stem (ES) cells which have been shown to generate progenitors for most hematopoietic lineages during differentiation *in vitro* (Keller, 1995) were also tested in this experiment. Figure 12C shows that *aa4* was not found in undifferentiated ES cells but its expression was upregulated as these cells differentiated and formed blast cell colonies (BL) and colonies of more differentiated hematopoietic cells (HMT). These results are in line with previous studies which showed that AA4 is expressed in ES-

derived hematopoietic precursors (Kabrun et al., 1997; Ling and Neben, 1997; Scott et al., 1997) and show that this expression is not abrogated upon differentiation of blast cell colonies into endothelial cells (ENT in Figure 12C).

Ectopic expression of AA4 has no mitogenic effect EML C1 and NIH 3T3 cells expressing exogenous AA4 did not exhibit morphological changes indicative of alterations in their growth properties. To examine the effect of AA4 on growth phenotypes in more detail, NIH 3T3 and primary mouse embryo fibroblasts were infected with a retrovirus expressing AA4 and maintained in high and low serum conditions. FACS analysis of the transduced cells confirmed that in each case the efficiency of infection was close to 100%. However, examination of growth rates showed that overexpressed AA4 had no apparent effect on proliferation of both cell types as compared to control uninfected cells or the corresponding cells infected with a retrovirus expressing GFP.

AA4 coimmunoprecipitates with CD34 and CD43 To investigate interactions with other proteins, hematopoietic and fibroblast cells expressing AA4 were immunoprecipitated with AA4.1 mAb and then examined by immunoblot analysis using a panel of antibodies directed against membrane-associated proteins. This analysis revealed that AA4 coimmunoprecipitates with CD34, a membrane glycoprotein selectively expressed within the hematopoietic system on stem and progenitor cells, and CD43 which is a major O-glycosylated sialomucin found on the surfaces of most leukocytes. This result is in line with previous studies which showed that CD43 coimmunoprecipitates with the human C1qRP (Guan et al., 1991; 1994). Figure 13B shows that in the murine D2N and EML C1 cells, a 52kDa protein is the major isoform of CD43 that associates with AA4. This 52kDa protein was found to be reactive with both the N-terminal (S19) and C-terminal (M19) anti-CD43 antibodies, indicating that it is not a breakdown product. In NIH 3T3 fibroblasts coexpressing CD43 and AA4, a 54kDa and a 170kDa CD43 isoforms coimmunoprecipitated with AA4 pointing to the glycosylation differences brtween CD43 expressed in different cell types. A 115kDa CD43 isoform which previously have been shown to be sialylated and thus overly negatively charged (Guan et al., 1994) did not form macromolecular complexes with AA4 in NIH 3T3 cells. Similarly, AA4 did not coimmunoprecipitate with a 115kDa CD43 isoform found in D2N cells and a 120kDa isoform found in EML C1 (Figure 13B).

To examine associations of AA4 with CD34, both proteins were expressed in Rat-1 cells following infection with the corresponding retroviruses. See Figure 13C.

In Rat-1 cells coexpressing AA4 and CD34, AA4.1 the results also demonstrate that AA4 coimmunoprecipitates with CD34. Studies have suggested a role for both CD34 and CD43 in the regulation of adhesion, growth and differentiation of hematopoietic precursors (Ardman et al., 1992; Bazil et al., 1997; Chen et al., 1996; Suzuki et al., 1996; Wood et al., 1997; Stockton et al., 1998). Coexpression of AA-Fc fusion protein in NIH 3T3 cells stably producing AA4 showed that AA4-Fc forms heterodimeric complexes with AA4. Figure 11A shows that equimolar amounts of AA4 and AA4-Fc were coprecipitated from these cells by protein A, indicating that AA4 is prone to homo- or heterodimerization.

Discussion

This Example describes the expression cloning and sequence analysis of AA4, a molecular marker expressed on hematopoietic stem and progenitor cells. The CDNA encoding AA4 was isolated from a retroviral cDNA library prepared from the murine D2N lymphoid cell line. Sequence analysis of the cloned cDNA revealed that AA4 is a type I transmembrane protein composed of 625 amino acids. The extracellular part of the molecule contains two major structural moieties, a C-type lectin carbohydrate recognition domain and six EGF-like domains. Similar repeats have been found in a large number of membrane-bound proteins or in proteins known to be secreted. The cytoplasmic domain of AA4, in contrast, bears no structural similarity with known protein families. Instead, AA4 revealed strong homology to ClqR, the human receptor for complement component Clq which is predominantly expressed in phagocytic cells such as monocytes, neutrophils, and endothelial cells (Nepomuceno et al., 1997). Sequence alighnment shows that AA4 and ClqR have approximately 68% identical amino acid positions and share similar domain structure. Highest homologies were found within the Nterminal parts of the two proteins and their C-terminal cytoplasmic domains, suggesting that AA4 and ClqR may have similar functional properties. Expression of AA4 correlates with the expression of CD34 and CD43, two other surface markers normally present on murine hematopoietic stem and progenitor cells. Previous studies have shown that AA4-positive cells are first detected in yolk sac at day 8-10 of gestation (Cumano et al., 1993; Sanchez et al., 1996).

At day 9-10 pc AA4+c-Kit+Mac-l+ progenitors are found in the P-Sp/AGM region (Sanchez et al., 1996; Marcos et al., 1997), and by day 14 of gestation, AA4

defines 0.5-1.0% of the fetal liver tissue that contains the entire hierarchy of primitive hematopoietic cells (Jordan et al., 1990). As proliferation within each successive compartment results in increased total number progenitor cells, antigen density per cell also increases with developmental progress, which is especially marked for c-Kit and AA4 (Marcos et al., 1997). A significant proportion of hematopoietic progenitors from yolk sac also express CD34, CD38, CD43, CD44, and Mac-1 however show little or no expression of CD4, CD8, CD45R and Sca-. These same markers are present on FLderived HSC which also express Sca-1. Adult marrow HSCs also express cKit, CD38 and Sca-1, but do not normally express Mac-I or AA4. In BM, HSC are found in both AA4+ and AA4-subpopulations, although in adult marrow AA4 is largely regarded to be a marker of early B lymphoid lineage 0. These results indicate that the expression of cell surface antigens changes on HSC during ontogeny and that differential display of theses cell surface markers may reflect relationship between HSCs that contribute to multilineage hematopoiesis and distinct anatomical sites during development.

Methods

Cells and tissue culture. NIH 3T3 fibroblasts were grown in DME medium supplemented with 10% fetal calf serum (FCS). D2N cells were grown in RPMI 1640 medium containing 10% FCS. EML Cl cells were grown in IMDM supplemented with 20% horse serum and 8% BHK/MKL conditioned medium (Tsai et al., 1994). To maintain the multipotentiality of EML Cl, the cells were kept at low density (0.5 - 5xlO-5/ml) and subcultured every two days. Cell lines constitutively expressing AA4 and GFP were derived from NIH 3T3 or EML Cl cells by infecting with the corresponding recombinant retroviruses.

Retroviral-mediated gene transler. Retrovirus expression vector REBNA was constructed by substituting the LacZ gene contained within the EcoRI-Notl fragment of plasmid LZRSPBMN-Z (Kinsella and Nolan, 1996) with a synthetic polylinker composed of EcoRl, Xhol, Sfil, and Notl sites. Retroviral vector REBNA/IRESGFP contains a poliovirus IRES element and the CDNA encoding color-enhanced GFP (S. Zolotukhin, Gainesville, FL) inserted into REBNA.

For DNA transfections, cells were plated at a density 2xlo6 cells per 60mm dish and transfected with 5ug of plasmid DNA using 20ul of lipofectamine reagent (GibcoBRL). REBNA-transfected cells were selected in puromycin (2ug/ml) and grown to confluence prior to collecting virus supernatant. For infections" the culture medium

was supplemented with polybrene (Sigma) at 5ug/ml. The appropriate virus was added and incubated overnight. Multiple *infections were carried* sequentially, with the *appropriate* selection between.

Isolation o.t RNA and CDNA cloning. Poly(A) RNA prepared from D2N cells was converted into CDNA using Superscript II Reverse Transcriptase (GibcoBRL) and an oligo(dt) primer containing NotI site, 5'-TGGTGTCGACGCAGAGTAGCGGCCGCT18 (SEQ.ID.No.:74). The second strand was synthesized using DNA polymerase I in combination with *E. coli* RNAse H and E. *coli* DNA ligase as described (Gubler and Hoffman, 1983). An adaptor composed of complementary oligonucleotides, 5'-GGCCCGGGCCGGCC (SEQ.ID.No.:75) and 5'-TCGAGGCCGCCCGGGCC (SEQ.ID.No.:76), was ligated to the CDNA and cut with Notl to produce CDNA molecules with NotI and XhoI termini for directional cloning. After size fractionation in agarose gel, cDNAs larger than 2.5 kbp were ligated into Notl and XhoI cut plasmid REBNA and electroporated into electrocompetent DH12S cells (GibcoBRL). Plasmid DNAs were transfected into 293-derived packaging cell line for retrovirus production. Virus-containing supernatants were collected and stored at -80C.

NIH 3T3 cells infected with the recombinant retroviruses representative of D2N CDNA library were selected for the production of AA4 by flow cytometry using phycoerythrin-conjugated AA4.1 mAb. After two rounds of sorting, genomic DNA isolated from AA4-positive cells was subjected to pcr amplification using retroviral vector primers, 5'CAGCCCTCACTCCTTCTC (SEQ.ID.No.: 77) and 5'-GGTGGGGTCTTTCATTCC (SEQ.ID.No.: 78) (Kitamura et al., 1995). Amplified CDNA was gel purified and subcloned into pbluescript SK and REBNA plasmid vectors. Nucleotide sequences were analyzed using NCBI Blast database search programs and ExPASy molecular biology server from the Swiss Institute of Bioinformatics.

Northern blot hybridization. RNAs prepared using acid guanidinium thiocyanate-phenol extraction procedure (Chomczynski and Sacchi, 1987) were separated.on formaldehydeagarose gels and blotted onto the Hybond-N nylon membranes (Amersham). Hybridization probes were derived from cloned cDNAs using Ready To Go DNA labeling beads (Pharmacia Biotech). Hybridizations were performed as described previously (Petrenko et al., 1997).

Cell labeling and Immunoprecipitations. The ECL protein biotinylation system (Amersham) for the detection of cell surface proteins was used as recommended by the

manufacturer. For radioactive labeling, 2xlo6 cells were incubated with 2OOuCi Translabel (ICN) in 2ml of cysteine and methionine-deficcient medium for 2hr at 370C. Cells were washed in PBS and lyzed 10min. on ice in 500ul of NP40 buffer containing 2OmM TrisHCI pH 7.6; l5OmM NaCl; 0.5% NP40; lmM PMSF; 5mM benzamidine; ImM sodium vanadate; lOug/ml aprotinin; lOug/ml leupeptin. Lysates were cleared by centrifugation and 2ug of soluble antibody were added to the supernatants followed by 25ul of Protein G-Sepharose suspension (Sigma). After 2 to 4hr incubation at 40C with rotation, protein Gantibody complexes were pelleted and washed in the successive changes of wash buffer I (lM NaCl; lOmM TrisHCI pH 8; 0.1% NP40); wash buffer 2 (O.lM NaCl; lOmM TrisHCI pH 8; 0.1% NP40); wash buffer 3 (lOmM TrisHCI pH 8; 0.1% NP40). Samples were eluted by boiling 2min. in 2Xgel loading buffer, separated by SDS/PAAG, dried and exposed to X-ray film.

Western blot analysis. Protein extracts for Western blot analysis were prepared as described (Morrison et al., 1991). The antibodies used included goat anti-mouse CD43 polyclonal IgG (Ml9 and S-19, Santa Cruz) in combination with HRPconjugated secondary antibodies and ECL detection system (Amersham).

Flow cytometry and RT-PCR. Timed-pregnant mice and 5- to 7-week-old female mice (C57BI/6j) were purchased from Jackson Laboratory (Bar Harbor, ME). AA4-positive cells were isolated from day 14 fetal liver by immunopanning on Petri dishes coated with AA4.1 antibody (10ug/ml). Hematopoietic stem cells were purified from AA4-positive fraction by staining with lineage-specific antibodies as described previously (Moore et al., 1997). Three-color fluorescence-activated cell sorting for lineage negative to low, Sca-l(+), c-Kit(+) cells was performed on a multilaser FACS Vantage with CellQuest software (Beckton Dickinson). ES cells differentiated into blasts cell colonies, hematopoietic progenitors, and endothelial cells prepared as described previously.

For RT-pcr, poly(A)-RNA isolated from sorted cells was converted into CDNA using Superscript II Reverse Transcriptase (GibcoBRL) and CapFinder CDNA amplification kit (Clontech). Gene-specific primers for pcr amplification included: 5'-TTCAGCAAGCCCTGACTC (SEQ.ID.No.:79) and 5'GCCACCTTCGAAGCAATC (SEQ.ID.No.:80) (AA4); 5'-GAGCGGTACAGGAGAATG (SEQ.ID.No.:81) and 5'GCCCACCCAACCAAATCA (SEQ.ID.No.:82) (CD34); 5'-ACCGCGTTCTTCTGTAAC (SEQ.ID.No.:83) and 5'CAGCTAACAGCAGGATCC

(SEQ.ID.No.:84) (CD43); G3PDH Control Amplimer Set (Clontech) for the amplification of GAPDH.

EXAMPLE 3: *IN VITRO* MAINTENANCE OF HIGHLY PURIFIED, TRANSPLANTABLE HEMATOPOIETIC STEM CELLS

The cellular and molecular mechanisms which regulate even the most primitive hematopoietic stem cell are not well understood. This example details a systematic dissection of the complex hematopoietic microenvironment in order to define some of these mechanisms. An extensive panel of immortalized stromal cell lines from murine fetal liver was established and characterized. Collectively, these cell lines display extensive heterogeneity in their *in vitro* hematopoietic supportive capacities. This example describes a long-term *in vitro* culture system, utilizing a single, stromal cell clone (AFT024) that qualitatively and quantitatively supports transplantable stem cell activity present in highly purified populations. Disclosed is multi-lineage reconstitution in mice that received the equivalent of as few as 100 purified bone marrow and fetal liver stem cells which were cultured for 4-7 weeks on AFT024. The cultured stem cells meet all functional criteria currently ascribed to the most primitive stem cell population. The levels of stem cell activity present after 5 weeks of coculture with AFT024 far exceed those present in short-term cytokine-supported cultures. In addition, the maintenance of input levels of transplantable stem cell activity is accompanied by the expansion of other classes of stem/progenitor cells. This suggests that the stem/progenitor cell population is actively proliferating in culture and that the AFT024 cell line provides a milieu which stimulates progenitor cell proliferation while maintaining *in vivo* repopulating activity.

Introduction

Mammalian blood formation originates in a small population of hematopoietic stem cells. The hallmark features of these cells are: (1) a hierarchical multilineage differentiation potential with the ability to clonally give rise to at least 8 distinct cell lineages, (2) self-renewal capacity which is reflected in the life long continuous activity of few, in some cases single, stem cells and (3) a dramatic proliferative potential which is ultimately responsible for the production of large numbers of mature blood cells. (Leminschka, I.R. 1992; Morrison, S.J. et al. 1995; Harrison, D.E. 1980). During the past decade much progress has been made in providing a physical phenotype for this rare population of stem cells.(Spangrude, G.J., et al. 1995; Lemischka, I.R. 1992; Bauman, J.G., et al. 1998). However, currently, the only reliable functional assay system for the most primitive stem cell compartment is long-term *in vivo* transplantation. No *in vitro* system has been

developed which adequately recapitulates stem cell behaviors. Therefore, the cellular and molecular mechanisms that regulate the biology of stem cells have remained obscure.

A major challenge in stem cell research is the establishment of culture systems which facilitate *in vitro* maintenance of long-term transplantable stem cell activity. This is a necessary first step towards a cellular and molecular understanding of the regulatory mechanisms which mediate commitment versus self-renewal decisions. Moreover, the establishment of such culture systems is a prerequisite for the potential expansion of undifferentiated stem cell populations as well as for the generation of stem/progenitor cells committed to selected lineages.

Efforts to develop culture systems for the maintenance of transplantable stem cells can be subdivided into two broad categories: (1) those utilizing defined cytokine combinations as the only culture supplements and (2) those relying on a pre-established stromal monolayer as an additional supportive component (with or without exogenously added cytokines). Both of these strategies have met with only limited success. In the first case, it has been repeatedly demonstrated that combinations of cytokines can exert potent stimulatory effects on stem/progenitor populations. (Spangrude, G.J., et al. 1988; Jordan, C.R. et al. 1990; Fleming, W.H., et al. 1993). In some studies, highly purified stem cells (Li, C.L., and Johnson, G.R. 1990; Spangrude, G.J. and Johnson, G.R. 1990). have been used and the direct effects of cytokines have been demonstrated at the single cell level. (Jones, R. et al. 1990; Uchida, N. et al. 1993). While informative, the vast majority of these studies are limited by their strictly *in vitro* nature. Thus, it is feasible to expand, replatable *in vitro* progenitor populations (Li, C.L., and Johnson G.R. 1992; Uchida, N. et al. 1993) and to stimulate colony-formation by cells with both myeloid-erythroid and lymphoid potentials, Jones, R., et al. 1990; Uchida, N. et al. 1993; Trevisan, M., and Iscove, N.N. 1995; Ogawa, M. 1993) however, the equivalence of these progenitor cells with the *in vivo* transplantable stem cell population remains speculative. Several studies have clearly demonstrated a dramatic loss of *in vivo* repopulating potential as a result of cytokine driven *in vitro* proliferation.(Knobel, et al., 1994; Peters, et al., 1995; Traycoff, et al., 1996). A small number of studies have shown that defined cytokine combinations promote the maintenance of transplantable activity.(Rebel., et al., 1994). However, most of these are limited both by the use of very short culture periods, the exact nature of the *in vivo* assay, and the use of non-enriched stem cell sources.(Muench, et al., 1993; Holyoake, et al., 1996; Soma, et al.,

1996). This precludes interpretations suggesting a direct action of the given cytokine(s) in maintaining transplantable activity.

A further complication with defined cytokine studies is the inability to ascribe *in vivo* physiological relevance to the observed effects. It has long been accepted that in the intact animal, stem cells are found in close association with discrete cellular microenvironments.(Lord, et al., 1975; Trentin, et al., 1970; Weiss, et al., 1991; Wolf, 1979). These observations suggest both the existence of stem cell niches and the notion that *in vivo* stem cell regulatory mechanisms are likely to require cell-cell contact or short range interactions.(Dorschking, 1990). Efforts to understand the features of the hematopoietic microenvironment began with the establishment of the Dexter long-term culture (LTC) system. (Dexter, et al., 1977). In this culture system hematopoiesis is maintained for weeks or months by a heterogeneous adherent cell monolayer derived from bone marrow (BM). While some degree of transplantable stem cell maintenance and self renewal (Fraser, et al., 1990) has been demonstrated, a general feature of the Dexter-LTC is a dramatic net decrease of stem cell activity over time. (Harrison, et al., 1987; Van der Sluijs, et al., 1993). Although much progress has been made, especially in studies of human stem/progenitor cells, (Sutherland, et al., 1989; Verfaillie, et al., 1995; Hao, et al., 1996) a further drawback of this system is the heterogeneity of the stromal cell types present in the supportive monolayer. This hampers the identification of regulatory mechanisms. Studies have been reported where the heterogeneous stromal monolayer is replaced with cloned stromal cell lines. (Roberts, et al., 1987; Kodama, et al., 1984; Issad, et al., 1993; Wineman, et al., 1993). Many of these cell lines can support *in vitro* myelopoiesis, (Suzuki, et al., 1992; Neben, et al., 1993; Kodama, et al., 1992) B-lymphopoiesis (Collins, et al., 1987; Whitlock, et al., 1982) or in some cases both.Wineman, et al., 1993; Wineman, et al., 1996) However, very few studies have focused on the *in vitro* maintenance of the most primitive transplantable stem cell compartment. Moreover, with one exception (Szilvassy, et al., 1996) the studies which have focused on the *in vitro* maintenance of this stem cell population begin with heterogeneous unpurified sources of hematopoietic activity. Wineman, et al., 1992, 1996; Deryugina, et al., 1994). Such populations contain numerous non-hematopoietic stromal cell types. Therefore, it has not yet been possible to assign a direct stem cell supporting phenotype to a given stromal cell line.

It was hypothesized that the rare frequency of primitive, stem cells may suggest an equally rare frequency of stem cell supporting microenvironmental niches. Accordingly, we

established and characterized a large panel of conditionally immortalized, cloned stromal cell lines from mid-gestation fetal liver. This organ was chosen because, during development, it is here that the stem cell compartment is undergoing self-renewal expansion in addition to differentiation. (Moore, et al., 1970). The cell lines were generated as previously described, (Wineman, et al., 1996) by immortalization with a temperature sensitive SV40TAg. (Frederiksen, et al., 1988) The clonal nature of the AFT024, 2018, and 2012 cell lines was verified by Southern blot analysis which detected a single, unique proviral integration locus in their genomic DNA. In order to identify potentially interesting cell lines, we used a "cobblestone area" (CA) assay (Ploemacher, et al., 1989) was initiated with BM taken from mice injected two days previously with 5-fluorouracil (5-FU). It has been suggested that CA colonies which appear after a prolonged culture period are derived from more primitive stem cells, possibly identical to some *in vivo* transplantable entities. (Ploemacher, et al., 1991). Therefore, a goal was in identifying cell lines which support such late arising CAs. Of 225 lines, 77 (34%) were capable of supporting limited *in vitro* hematopoiesis, while, consistent with the initial hypothesis, only 2% were able to maintain long-term (>6 weeks) hematopoietic CA activity. Subsequent studies with a selected subset of these lines, showed that the ability to effectively support *in vivo* reconstituting BM stem cells is infrequently observed. (Wineman, et al., 1996). Two out of sixteen cell lines maintained significant levels of long-term reconstituting stem cell activity for an *in vitro* culture period of three weeks. Several other cell lines supported low levels of such activity or transiently repopulating stem cells. The cell inoculum, in these studies, was whole BM which was not enriched for stem cell activity. Therefore, it was not possible to suggest that the effective stromal cell lines were directly supporting stem cell activity. This example demonstrates that a single clonal cell line, designated AFT024, can maintain quantitative levels of transplantable stem cell activity present in highly purified stem cell populations. These data were generated using a competitive repopulation assay system, which employs uncompromised competitor BM cells. The *in vitro*-maintained stem cells satisfy all criteria which currently define the most primitive stem cell population including the ability to reconstitute secondary recipients. This example also shows that the *in vitro* maintenance of primitive transplantable stem cells is compatible with the concurrent generation of large numbers of committed progenitors.

Methods

Mice. Timed-pregnant mice and 5-7 weeks old females (C57Bl/6J, Ly5.2) were purchased from the Jackson Laboratory (Bar Harbor, ME). Congenic C57Bl/6, Ly5.1 female mice were purchased from the National Cancer Institute (Frederick, MD). All mice were housed in the Princeton University Barrier Animal Facility, in autoclaved micro-isolator cages on ventilated cage racks. The animals received sterile, irradiated food, and acidified, autoclaved water ad libitum.

Stromal cell lines and culture conditions. The fetal liver stromal cell lines used in this study were derived as previously described. (Wineman, et al., 1996). Stromal cell lines were routinely cultured in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), $5X10^{-5}$ mol/L b-mercaptoethanol (2-ME), at 32^0C, 5% CO_2, 100% humidity. Sera were obtained from Hyclone, Logan, UT. Other biochemical reagents were obtained from Sigma, St. Louis, MO. Two of the lines used in this study (2012 and 2018) were previously characterized for their ability to support long-term repopulating activity present in whole unfractionated BM. (Wineman, et al., 1996). The AFT024 cell line was identified as an additional long-term (>4 weeks) CA supporter. Subclones of 2012 and AFT024 were isolated and used in these studies. The AFT024 cell line has remained stable and demonstrated consistent stem cell supporting abilities for over 4 years.

Hematopoietic stem cell purification. Stem cells were purified from day 14 fetal livers essentially as described, (Jordan, et al., 1990) with the inclusion of c-kit expression as an additional parameter. Briefly, $AA4.1^+$ cells were isolated by immunopanning on petri dishes coated with AA4.1 antibody (10 ug/mL). The $AA4.1^+$ fraction has been shown to contain all repopulating stem cell activity present in day 14 fetal liver. (Jordan, et al., 1990) $AA4.1^+$ cells were collected and stained with saturating concentrations of fluorescein isothiocyanate (FITC) labeled rat monoclonal antibodies to lineage markers (CD3, CD4, CD5, CD8, B220, Gr-1, Mac-1, and TER-119). The cells were simultaneously stained with phycoerythrin (PE) labeled Ly6A/E (Sca-1) antibody and biotinylated antibody to c-kit. The latter was developed with streptavidin allophycocyanin (APC). The AA4.1 hybridoma was a kind gift from Dr. J. McKearn, Monsanto, St. Louis, MO. AA4.1 antibody was purified by ImClone Systems Inc. New York, NY. The TER-119 antibody was initially obtained from Dr. T. Kina, Kyoto University, Japan and subsequently purchased from PharMingen, San Diego, CA. All

other antibodies were purchased from PharMingen. Streptavidin APC was purchased from Molecular Probes Inc., Eugene, OR. Three color fluorescence activated cell sorting for lineage negative to low ($lin^{-/lo}$), Sca-1^+, c-kit^+ cells was initially done on a dual laser Epics 753 cell sorter (Coulter Electronics, Hialeah, FA) interfaced with Cicero software (Cytomation Inc., Fort Collins, CO) and subsequently on a multi-laser FACS Vantage with Cell Quest software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Enriched fractions of BM stem cells were obtained from C57Bl/6 Ly5.1 mice as described. (Okada, et al., 1992). Briefly, BM mononuclear cells were isolated by density centrifugation over Ficoll Hypaque (<1.077, Pharmacia, Piscataway, NJ). Lineage negative or low staining cells ($lin^{-/lo}$) were obtained by magnetic bead depletion (anti-rat immunoglobulin coated beads, Dynal, Oslo, Norway) of mononuclear cells using the same lineage cocktail described herein above. The cells were further stained with antibodies to Sca-1 and c-kit as described herein above. Sorting for $lin^{-/lo}$, Sca-1^+, and c-kit^+ cells was accomplished with the Epics 753 as described above.

Stem cell/stromal cell cocultivation and cytokine-supplemented suspension culture. Stromal cell lines were seeded on tissue culture dishes that had been coated with 1% gelatin (Specialty Media, Lavallette, NJ) and were grown at 32^0C, 5% CO_2, 100% humidity. Confluent monolayers were irradiated (20 Gy, ^{137}Cesium source, Gammacell 40, Nordion International Inc. Ontario, Canada) and cultured in modified Dexter (Dexter, et al., 1984) media (DMEM, 10% FBS, 10% horse serum, $5X10^{-5}$ mol/L 2-ME, $1X10^{-7}$ mol/L hydrocortisone). For Dexter-LTC, enriched hematopoietic stem cells were added and the cultures were maintained at 37^0 C, 5% CO_2, 100% humidity with weekly media changes. The specific numbers of purified stem cells added to stromal cell cocultures are given in the appropriate figure or table legends. In some experiments, week 4 AFT024/stem cell cultures were harvested and replated in limiting-dilution onto fresh, irradiated (20 Gy) AFT024 monolayers in 96-well trays (Dexter-LTC conditions). CAs were scored weekly (as described above), for an additional 5 weeks. Irradiated (20 Gy) 2018 monolayers in 96-well trays were used in limiting-dilution Whitlock-Witte assays (LD-WW) (Whitlock, et al., 1982) to assess stromal-dependent B-lymphopoiesis content of both freshly purified and AFT024 cultured fetal liver stem cells. These cultures were established in RPMI media with 5% FBS, 2 mmol/L glutamine, 1 mmol/L Na pyruvate, and $5X10^{-5}$ mol/L 2-ME at 37^0 C, 5% CO_2, 100% humidity. 2018 has been identified as a potent B-lymphopoiesis supporting line in W-W conditions. (Deryugina, et al., 1994).

Short-term cytokine-supported suspension cultures and short-term AFT024/stem cell cocultures were established in Iscove's Modified Dulbecco's Media (IMDM), 10% FBS, 1% BSA, $5X10^{-5}$ mol/L 2-ME at 37^0C, 5% CO_2, 100% humidity. Cytokine concentrations: rmflk2/flt3-ligand (FL) 30 ng/mL, rmSteel factor (SL) 20 ng/mL, rhIL-6 10 ng/mL. FL was obtained from ImClone Systems Inc.; SL was purchased from Genzyme Corporation, Cambridge, MA. IL-6 was purchased from Upstate Biotechnology Inc., Lake Placid, NY.

Transplantation assays for hematopoietic stem cell activity. Competitive repopulation was used to measure stem cell activity present in both freshly isolated and cultured stem cell populations. (Harrison, et al., 1993). This assay was performed using the congenic Ly5.1/5.2 mouse system (Morse, et al., 1987). Enriched stem cells were seeded onto irradiated stromal monolayers and maintained in Dexter-LTC conditions. At the end of 4-7 weeks, the cultures were harvested by vigorous trituration. Single cell suspensions were prepared by passage through 22-gauge needles, mixed with fresh congenic BM and transplanted into lethally irradiated congenic mice (10 Gy, split dose 3 hours apart, 1 Gy/min, Gammacell 40). All purified fetal liver stem cells were from Ly5.2 mice. The competitor BM and recipients were Ly5.1. Purified BM cells were from Ly5.1 mice; in this experiment, Ly5.2 BM was used as competitor and Ly5.2 mice were used as recipients. In order to assess reconstitution, mice were periodically bled by capillary puncture of the orbital venous plexus. Blood (0.1 mL) was collected into heparin-containing (10 U/mL) DMEM and the red blood cells were lysed with NH_4Cl (Mishell, et al., 1980). For the experiments described in Figure 15 and Table 4., the nucleated cells were divided into two fractions and stained with the appropriate biotinylated Ly5 antibody and developed with streptavidin-peridinin chlorophyll protein (PerCP) and; (1) CD4-FITC, CD8-PE and (2) B220-FITC, Mac-1-PE, Gr-1-PE. Cells from each fraction were analyzed on an Epics Profile II, Coulter Electronics. For the experiments described in Figure 16 and Tables 2. and 3., nucleated cells were stained with directly conjugated lineage antibodies (CD4-PE, CD8-PE, Mac-1-FITC, Gr-1-FITC, and B220-APC) and biotinylated Ly5.2 antibody which was developed with streptavidin Texas Red (T.R.). Four color analysis of stained cells was performed on either the Coulter Epics 753 or Becton Dickinson FACS Vantage with the appropriate software interfaces described above. Anti-Ly5.1 was a kind gift of Dr. H. Nakauchi, University of Tsukuba, Japan. Purified and biotinylated Ly5.2 antibody was originally obtained from a hybridoma

(AL14A2) kindly provided by Dr. G. Spangrude, University of Utah Medical Center, Salt Lake City, UT. In later experiments, the Ly5.2 antibody was purchased from PharMingen; CD4-PE, CD8-PE, and B220-APC were also purchased from PharMingen. Streptavidin-PerCP was purchased from Becton Dickinson Immunocytometry Systems. Streptavidin T.R. was purchased from Molecular Probes. Competitive repopulating units (CRU) per 10^5 were calculated according to the formula: (Harrison, et al., 1990).

$$CRU/10^5 = \frac{\%Ly5\ positive\ cells}{100-\%Ly5} \times \frac{cell\ number\ competitor\ BM}{number\ of\ test\ cells}$$

Reconstitution values of less than 2% of the test Ly5 donor allele were not considered sufficiently above background for calculation of CRU.

Retransplantation potential of stem cells was assessed by secondary transplantation. Mice from the experiment presented in Figure 14. were sacrificed 60 weeks after transplantation, BM was harvested and stained with antibody to Ly5.2 followed by streptavidin T.R. $Ly5.2^+$ cells were collected by cell sorting (Coulter Epics 753) and used to transplant lethally irradiated secondary Ly5.1 recipients. Marrow from mice in the Control and AFT024 groups was used to transplant mice in both radioprotection and competitive repopulation assays. $Ly5.2^+$ BM from the primary 2012 transplants was used only in radioprotection assays. Primary 2018 mice did not contain sufficient $Ly5.2^+$ cells for secondary transplantation. Transplanted mice were bled and analyzed by 4-color flow cytometry for the presence of $Ly5.2^+$ cells and multilineage reconstitution as described above.

In vitro hematopoietic progenitor cell assays. The progenitor content of freshly purified hematopoietic stem cell populations and AFT024/stem cell cocultures was assessed using a variety of *in vitro* assays. All of the following assays were accomplished with fetal liver stem cells enriched as described herein above. To determine the time course of CA development, enriched stem cells were seeded onto irradiated AFT024 monolayers (300-600 cells/well in 12-well trays). CA development was followed over time and characteristic clusters were quantitated as described above. At different time points of the stem cell/AFT024 cultures, individual wells were harvested and replated into cytokine-supplemented semisolid clonogenic progenitor assays (CFU-C). The cytokine-enriched (rmIL-3 10 ng/mL, rhIL-6 10 ng/mL, rmSL 50 ng/mL, Epo 3 U/mL) methyl-

cellulose mixture was purchased from Stem Cell Technologies Inc., Vancouver BC, Canada. Colonies were scored after 8-14 days of culture at 37^0, 5% CO_2, 100% humidity according to established criteria. (Testa, et al., 1993). Colonies that reached >1mm in size after 8 days and which contained erythroid bursts and multiple myeloid cell lineages including megakaryocytes were scored as high-proliferative potential-mixed lineage colonies (CFU-HPP-Mix). (Lowry, et al., 1995). Lineage content of typical colonies was determined by Wright's/Giemsa staining of cytospin slide preparation from individual colonies. Colony assays were also done with 10^3 freshly purified cells. The CFU progenitor contents of the AFT024 cocultures were normalized to an initial input of 10^3 stem cells. To assess the ability of the AFT024 cell line to maintain primitive lymphoid progenitors, 4 week cocultures were plated into LD-WW assay on 2018 cells as described above. Resulting pro-B cell colonies were scored after 7 days. The cell number in individual wells (96-well trays, 8 wells/cell number) was normalized from the original number of purified stem cells that initiated the coculture, i.e. stem cell equivalents/well. As calculated from the line of best fit, the cell number at 37% negative wells is the frequency of pro-B cell colony initiating cells in the starting population. (Taswell, 1981). In a similar manner, the frequency of CA initiating cells in week 4 stem cell/AFT024 cocultures was also determined by replating them in limiting-dilution onto fresh, irradiated AFT024 monolayers in 96-well trays. CA were scored as described above, at 1, 2, 3, 4, and 5 weeks after replating in Dexter-LTC. The resulting frequencies were calculated as described herein above for the LD-WW assays and are also expressed in relationship to the number of stem cells that seeded the initial cultures (stem cell equivalents).

Results

In vivo and in vitro assays for stem cell activity maintained by stromal cell lines. Highly enriched stem cell populations were used to initiate cultures supported by single stromal cell lines. The focus was on one cell line, AFT024, which exhibited particularly potent stem/progenitor cell support. Two other stromal cell lines, 2012 and 2018, (Wineman, et al., 1996) were included in some experiments. In order to more rigorously establish the clonality of these lines, they were subcloned by limiting-dilution. All subclones obtained from a given cell line contained the same proviral integrant position as the parental cell line. The AFT024 cell line was evaluated both for its ability to maintain *in vivo* competitive repopulating stem cells, as well as a broad spectrum of

stem/progenitor cells defined by a variety of *in vitro* assays. The *in vivo* assays focused on the ability of stem cells, cultured for extended time periods (4-7 weeks), to permanently reconstitute multi-lineage hematopoiesis in transplanted hosts. The *in vitro* assays included the enumeration of CA appearing over time in the initial cultures as well as the quantitation of stem/progenitor cells which can form colonies in cytokine-supplemented replating assays. Cells from four-week AFT024/stem cell cocultures were also assayed by limiting-dilution for the content of progenitors capable of initiating secondary CAs on AFT024 or B-lymphopoiesis in Whitlock-Witte cultures supported by 2018.

AFT024 maintains quantitative levels of long-term in vivo repopulating stem cell activity. One line of investigation inquired if and at what levels *in vivo* transplantable stem cell activity was present in four to seven week-old cultures initiated with highly enriched stem cells and supported by AFT024, 2012 or 2018. Purified day 14 fetal liver cells ($AA4.1^+$, $lin^{-/lo}$, $Sca\text{-}1^+$, $c\text{-}kit^+$) and adult BM cells ($lin^{-/lo}$, $Sca\text{-}1^+$, $c\text{-}kit^+$) were used as sources of stem cell activity. Both of these populations are about 1000 to 1500-fold enriched for stem cell activity, as measured by competitive repopulation. (Harrison, et al., 1993). The Ly5.1/Ly5.2 congenic system was utilized for all competitive repopulation studies. (Morse, et al., 1987). The data presented in Figure 14 demonstrate that the cultures supported by AFT024 contain stem cell activity at levels quantitatively identical to those present in the uncultured purified populations. In this experiment, individual Ly5.1 mice received 10^3 freshly purified Ly5.2 cells or the cultured equivalent of 10^3 purified Ly5.2 cells. Each mouse also received 10^6 Ly5.1 competitor BM cells. The percentage of Ly5.2 positive peripheral blood cells was approximately equal in both groups of recipient animals. Moreover, the cultured stem cell activity is as effective as freshly purified activity for *in vivo* periods of greater than one year. The data in Figure 14 also show that the 2018 cell line is completely ineffective in maintaining highly purified stem cell activity while the 2012 cell line supports intermediate levels of repopulating activity. The data presented in Table 4A. provide quantitative competitive repopulating unit (CRU) value calculations as well as the results of multiparameter lineage analyses. The extremely low levels of reconstitution by 2018-cultured stem cells precluded lineage analysis. The CRU values of the AFT024-cultured and freshly purified populations are nearly identical. Moreover, both fresh and AFT024-cultured stem cells reconstitute myeloid and lymphoid cell populations to a similar degree. In order to further access the

supporting activities of AFT024 and 2012 we utilized a 10-fold lower number of fetal liver stem cells from two separate purifications to initiate the cocultures. The cultures were continued for 4-7 weeks, harvested, and used in competitive repopulation studies. Each recipient received the cultured equivalent of 100 purified Ly5.2 stem cells plus $4X10^5$ Ly5.1 competitor BM cells. A total of twelve mice were transplanted with AFT024 cocultures (four each after four, five and seven weeks of LTC). The parental AFT024 line was used in the 4 week group and two different subclones were used to support the five and seven week cultures. The AFT024-cultured Ly5.2 stem cells contributed to 20-30% of peripheral blood cells in these recipients while cells cultured on 2012 demonstrated more limited *in vivo* function (Table 4B). The 2012 cultures were done with two subclones of the parental line and were maintained for four weeks prior to harvest and transplant (four mice/subclone). The data utilizing different cultures time or sublcones did not vary significantly from each other and are presented together in Table 4B. An additional experiment was undertaken using enriched BM which was cultured on AFT024 and 2018 for six weeks. In this experiment, BM was purified from Ly5.1 congenic mice. Each Ly5.2 recipient mouse in this study received 100 freshly purified cells, or the cultured equivalent of 100 purified cells. Both groups received 10^5 Ly5.1 competitor BM cells per mouse. Data analysis, for the presence of $Ly5.1^+$ cells at 4 months after transplant, is presented in Table 4C. For 6 weeks of culture, AFT024 cells maintained quantitative levels of reconstituting activity present in 100 purified BM stem cells. The 2018 cell line failed to maintain stem cell activity.

These studies were extended to include secondary transplantation as an additional assay for primitive stem cells. BM cells were harvested from the primary recipients of fresh and cultured fetal liver stem cells (see Figure 14. and Table 4A.) and the Ly5.2 positive, fetal liver-derived fraction was collected by cell-sorting. Secondary radioprotection and competitive repopulation transplants were performed. The data are presented in Table 5. The secondary recipient repopulating activities are nearly identical for the AFT024 cultured stem cells and the non-cultured controls. Lineage analysis of the Ly5.2 cells in the secondary recipients revealed similar numbers of myeloid and lymphoid cells derived from both AFT024-cultured and non-cultured stem cells. Some level of secondary reconstituting cell activity was observed.

Additional experiments were also performed to determine where the levels of stem cell activity present in long-term AFT024 cocultures were compared to those present

in short-term cytokine stimulated cultures or in short-term AFT024-supported cultures (Figure 15). Purified fetal liver cells were seeded onto an AFT024 monolayer and maintained in Dexter-LTC conditions for 5 weeks. Simultaneously, the same numbers of purified cells were cultured for 5 days with; (1) different cytokine combinations or (2) on AFT024. The transplantable activity in the cultured cells was then assayed by competitive repopulation. Each mouse received the cultured equivalent of 600 stem cells together with $4X10^5$ Ly5.1 competitor BM cells. It is evident from the data that the levels of *in vivo* repopulating activity present in the long-term AFT024-supported cultures are much greater than those remaining after a short-term cytokine-supported culture period. Of interest also is that short-term AFT024 stem cell cocultures do not maintain significant *in vivo* reconstituting activity. In fact, these levels of stem cell activity are identical to the levels seen in the cytokine-supported cultures.

In vitro stem/progenitor populations are expanded by AFT024. AFT024/stem cell cocultures have vigorous hematopoiesis throughout the entire *in vitro* culture period. This is reflected in the large numbers of relatively mature hematopoietic cells which are produced throughout the culture period. In addition, CA colonies are observed throughout the culture period. Figure 16 shows a time course of CA appearance with purified fetal liver stem cells (3 separate experiments). After 28 days in culture, approximately one in every twenty input stem cells is capable of proliferating into a CA. In addition, CA appearance over time follows a biphasic distribution, with many CA observed early in the culture period. In order to enumerate the various classes of stem/progenitor cells present in AFT024 cocultures, we performed a series of *in vitro* replating experiments. These included the quantitation of: (1.) progenitor cells capable of colony-formation in cytokine-supplemented semisolid cultures (CFU assay), (2.) progenitor cells capable of initiating secondary CA in limiting-dilution AFT024 cultures and (3.) progenitor cells which can initiate B-lymphopoiesis in LD-WW cultures. In all of these experiments the primary cultures were initiated with purified fetal liver cells. All data presented below are normalized to an initial input of 10^3 purified cells (CFU assay) or the actual number of initial input stem cells (limiting-dilution assays).

Shown in Figure 17 are the numbers and types of cytokine responsive CFU progenitors present at various times in the AFT024-supported cocultures. Production of CFU is evident at all time points. However, the content is especially high after four weeks, representing a 5-7 fold increase/expansion when compared to the content in the

freshly purified populations. The content of more primitive progenitors (CFU-HPP-Mix) is increased by 12-fold. These HPP-Mix colonies often reach a size of 2 mm in 8 days and contain large numbers of erythroid bursts and megakaryocytes. Interestingly, there does not appear to be a correlation between CA number and CFU content at different culture times. This is most apparent at day 6, when CA numbers are at their peak but the progenitor content is similar to that observed in non-cultured stem cells. Furthermore, there is no correlation between CFU content and the absolute numbers of maturing hematopoietic cells present in a given culture.

Next, the content of primitive B-lymphoid progenitors present in the AFT024/stem cell cultures was determined. This was accomplished by plating cells from the four-week AFT024 cocultures into LD-WW assays over the 2018 stromal cell line. Two experiments with freshly purified stem cells and AFT024-cultured stem cells showed that the frequency of pro-B cell progenitors is expanded 10-fold in AFT024 cultures compared to the frequency observed in the freshly purified input population (day 0 frequency 1 in 11.0, r^2= 0.98; day 28 AFT024-cultured frequency 1 in 1.1, r^2= 0.97).

In order to measure the content or frequency of progenitor cells capable of initiating secondary CA, 4 separate, four-week AFT024/stem cell cocultures were replated in limiting-dilution onto fresh AFT024 monolayers. CAs were scored after one week. The data are presented in Figure 18A. Large numbers of secondary CAs were observed. When normalized to the stem cell numbers used to initiate the primary cultures (stem cell equivalents, see Methods), the frequency of these progenitors is 1 in 3 to 4. Figure 18B. shows data from one of the 4 experiments presented in Figure 18A., where the quantitation of secondary CAs was extended for 4 more weeks. The frequency of CA decreases slowly over time (1 in 19 after an additional 4 weeks), approximating the frequency seen in the primary cultures at four weeks. In summary, our *in vitro* replating assays collectively demonstrate a significant expansion of primitive progenitor populations in 4 week AFT024 cultures. In these same cultures there is no decrease in the levels of transplantable stem cell activity present in the total hematopoietic cell population.

Discussion

In this example, it was demonstrated, that the AFT024 stromal cell line can maintain quantitative levels of *in vivo* repopulating stem cells for at least 7 weeks of *in vitro* culture. Highly enriched stem cell populations in low numbers (100 cells) were

used and cell activity was measured in a stringent, competitive repopulation assay system. The cultured stem cell activity satisfies all *in vivo* criteria normally ascribed to the most primitive stem cell compartment; (1) long-term engraftment ability, (2) multilineage potential and (3) the ability to repopulate secondary recipients. In addition, the studies with the low culture initiating stem cell numbers for both BM and fetal liver, imply that AFT024 stromal cells exert their supportive effects in a direct manner. These studies represent a clear example of an *in vitro* system capable of directly supporting the most primitive stem cell compartment.

Other data revealed that stromal cell lines isolated from a single tissue source are heterogeneous with respect to their abilities in maintaining long-term repopulating stem cells. (Wineman, et al., 1996). It was speculated that the rare cell lines which were effective in supporting *in vivo* reconstituting stem cells may represent immortalized components of *in vivo* stem cell niches. However, the data argue for the necessity of using purified stem cell populations in order to support such a hypothesis. Specifically, the 2018 cell line maintained transiently reconstituting activity present in unfractionated BM. However, 2018 fails to maintain measurable repopulating activity when cultured for 4-6 weeks with highly purified BM or fetal liver stem cells. Similarly, in experiments using purified cells, there was a failure to show robust levels of reconstituting stem cell activity in cultures supported by CFC034, the most effective cell line in the whole BM studies (Wineman, et al., 1996). The 2012 cell line which was reported (Wineman, et al., 1996) to be effective in maintaining long-term repopulating whole BM derived stem cells is only partially effective in the present studies. Moreover, only some subclones of 2012 display such activity (in spite of identical proviral integration positions in all subclones). Recently, studies have shown that the S17 cell line which consistently supports the stem cell activity present in whole BM, (Wineman, et al.,. 1993) is not similarly effective in the maintenance of purified BM stem cells. (Szilvassy, et al., 1996). Taken together with these current data, the previously observed stem cell supporting stromal cell activities may reflect the action(s) of indirect mechanisms and therefore do not permit the identification of cellular stem cell niche components. One previous study has shown that the Sys1 stromal cell line can maintain high levels of transplantable activity present in purified BM. (Szilvassy, et al., 1996). The competitor cells in that study were compromised by prior serial transplantation. Moreover, the culture period was extended for only two weeks and effective maintenance required the addition of exogenous

leukemia inhibitory factor. In contrast, AFT024 is a cell line that provides a direct-acting long-term stem cell supporting environment without the addition of exogenous factors.

In addition to recovering net input levels of transplantable activity from AFT024 supported cultures, significantly expanded populations of primitive progenitor cells were also obtained. CFU-HPP-Mix progenitors are expanded by 12-fold after 4 weeks of culture and the numbers of stromal-dependent pro-B lymphoid progenitors are similarly amplified. This suggests that the AFT024-mediated process of stem cell maintenance is in reality a dynamic phenomenon. Specifically, during the first portion of the culture period, the majority of transplantable stem cell activity may be lost, through differentiation or cell death. The remaining primitive stem cells may expand to yield input levels of transplantable activity as well as increases in the numbers of more committed progenitors. One hypothesis is that short-term AFT024 supported cultures should contain reduced levels of transplantable stem cell activity. Figure 17 support this hypothesis. The standard Dexter-type media used in parallel long-term AFT024 cocultures was not used in these short-term cultures. However, in another short-term experiment, utilizing Dexter-LTC media, a similarly dramatic reduction in stem cell activity was observed after 4 days of culture on AFT024.[63] These observations are intriguing because they suggest that the AFT024 cell line is able to facilitate some degree of *ex vivo* transplantable stem cell proliferation and expansion. Indeed, in other studies it was shown that AFT024 can support colony formation initiated by single purified stem cells with B and T-lymphoid, myeloid and erythroid potentials. Moreover, the data suggest that is possible to efficiently introduce retroviral markers into transplantable stem cells at various times during AFT024 cocultures. Extension of such marking experiments and an analysis of proviral integration patterns will be necessary to rigorously ascertain if self-renewal replication is occurring during these coculture periods.

The ability of AFT024 to maintain the most primitive stem cell compartment while generating and expanding at least some less primitive members of the stem/progenitor cell hierarchy raises interesting issues regarding the nature of stem cell niches. The present invention suggests that microenvironmental niche models which postulate distinct cellular entities responsible for stem cell self-renewal and other cellular entities which support the generation of committed progenitor cells may be overly simplified. (Uchida, et al., 1993). Quite clearly a single microenvironmental cell type represented by AFT024 is sufficient for keeping stem cells in an undifferentiated state as

well as allowing commitment and progenitor expansion to take place. A hallmark feature of a stem cell niche is the ability to facilitate the generation of the entire stem/progenitor cell hierarchy from very primitive cells. Therefore, the main functional role of such niches may be to provide an environment which permits the production of the correct numerical balance of more and less primitive stem/progenitor cell entities. This model contains several testable hypotheses. The most important is that *in vitro* stem cell maintenance should not be interpreted, literally, as the maintenance of quiescent cells but rather as a phenomenon which results from a balance of self-renewal and commitment decisions which occur during stem cell division.

A cytokine cocktail of IL-6, SL, and FL is not effective in maintaining fetal liver stem cell activity. It was shown that RNA transcripts for these and 10 other cytokines are present in AFT024, but they are also detected at similar levels in non-supporting lines such as 2018. (Wineman, et al., 1996). These observations suggest the existence of novel AFT024-derived molecules which may act on stem cells. Indeed, using a subtractive hybridization molecular cloning strategy, a number of candidate molecules have been identified. Two of these molecules contain EGF-like repeat motifs that are most closely related to those found in the Notch/Notch-ligand family. Interestingly, one of these molecules appears to have activity on primitive stem cell populations. (Moore, et al., 1997). (See, Examples presented herein above.

Table 4. Multilineage stem cell activity, high CRU levels maintained in AFT024 cultures

A. With 1000 purified fetal liver cells

		Lineage Contribution, % Ly 5.2 Cells				Total	
Cells	Week	CD4	CD8	B220	Myeloid	% Ly 5.2	CRU/10^5
Control	5	3.3±1.5	4.4±1.7	48±5.1	32±6.0	28±1.3	386±24
	12	27±10	24±8.7	63±8.9	43±9.2	44±3.2	800±102
	24	42±6.9	34±4.1	71±4.4	62±1.4	48±4.8	984±171
AFT024	5	7.7±6.2	6.0±3.5	58±6.6	36±4.7	39±2.1	641±54
	12	44±7.1	38±6.4	70±5.6	53±3.6	48±1.4	942±51
	24	56±3.8	49±3.1	72±2.4	64±3.7	56±1.9	1270±96
2012	5	0.8±0.5	1.4±0.3	6.8±3.6	14±11	7.4±2.3	52±8.5
	12	9.1±8.4	10±6.9	14±9.3	23±20	14±4.5	194±68
	24	17±3.8	18±2.0	23±5.9	32±6.4	21±4.4	196±71

B. With 100 purified fetal liver cells

		Lineage Contribution, % Ly5.2 Cells			Total	
Cells	Week	Granulocytes	B-cells	T-Cells	% Ly5.2	CRU/10^5
AFT024	15	33±4.6	41±6.0	25±5.3	28±4.6	1888±315
	26	17±4.4	18±4.8	18±5.9	24±5.9	1825±571
	46	22±5.7	19±6.6	15±6.7	20±6.5	1595±628
2012	15	32±7.8	28±7.2	20±5.2	24±6.6	1390±382
	26	10±2.8	9.7±3.2	9.7±3.2	14±3.9	699±176
	46	11±2.3	6.7±1.6	8.9±3.2	9.0±2.0	409± 80

C. With 100 purified bone marrow cells

	No. of	Lineage Contribution, % Ly 5.1 Cells				Total	
Cells	mice	CD4	CD8	B220	Myeloid	% Ly 5.1	CRU/10^5
Control	8	38±7.3	30±5.8	43±6.7	31±4.8	32±6.5	583±172
AFT024	7	20±9.8	18±8.8	20±8.7	24±11	22±10	490±296

A. AA4.1$^+$ day 14 fetal liver cells (Ly 5.2) were further purified for a lin$^{-/lo}$, Sca-1$^+$, ckit$^+$ stem cell surface phenotype. Fresh purified control cells, 10^3, were transplanted with 10^6 Ly5.1 competitor marrow (n=6 mice). From the same purification, 10^4 cells were cocultured with stromal cell lines for 4 weeks. Subsequently, 10% of each culture was transplanted per mouse (n=8 mice/stroma) together with 10^6 competitor BM cells. The contribution to each lineage in peripheral blood is expressed as the percent of the total specific lineage population that was Ly5.2$^+$. CRU/10^5, relative enrichment of competitive repopulating units. Data are presented ±SEM.

B. The lineage and CRU content of low numbers of enriched fetal liver stem cells maintained on AFT024 and 2012 were determined. 500 stem cells were maintained in Dexter-LTC for 4-7 weeks over irradiated monolayers. 20% of each culture was used to transplant groups of 4 mice (i.e. each mouse received the equivalent of 100 stem cells that initially seeded the cultures) combined with 4X10^5 competitor BM cells. Data are presented as ±SEM.

C. Ly 5.1 BM cells with a lin$^{-/lo}$, Sca-1$^+$, and c-kit$^+$ cell surface phenotype were purified. 100 fresh cells per mouse (Control) were transplanted with 10^5 Ly 5.2 competitor BM cells. One thousand of the same purified cells were cocultured with stromal cell lines for 6 weeks. The cultures were then harvested and 10% of each culture was transplanted per mouse with competitor. Data are from peripheral blood samples taken 4 months after transplant and are presented ±SEM.

Table 5. LTRSC maintained on AFT024 are able to repopulate secondary recipients at levels comparable to non-cultured stem cells.

Group	Weeks	% Ly 5.2^+ peripheral blood cells	
		Radioprotection	Comp. Repopulation
Control	6	21 (1)	2.6±0.6 (4)
	22	54 (1)	5.6±1.6 (4)
AFT024	6	13±0.2 (4)	1.3±0.9 (8)
	22	44±15.1 (3)	4.3±0.9 (8)
2012	6	6.7±2.9 (4)	ND
	22	14±2.2 (4)	ND

The retransplantation potential of LTRSC in primary recipients of stromal cell cultured stem cells was studied in secondary recipients. 60 weeks after transplant, primary mice (see Figure 14, Table 4A.) were sacrificed, BM harvested and stained with antibody to Ly5.2. $Ly5.2^+$ cells were collected by cell sorting and used to transplant secondary recipients (congenic Ly5.1 mice). Control and AFT024 groups were transplanted with $1.5X10^6$ Ly5.2 cells/mouse for radioprotection (4 mice/group were transplanted) and $7.5X10^5$ Ly5.2 cells + $7.5X10^5$ Ly5.1 cells for competitive repopulation (4 mice for the Control group and 8 mice for the AFT024 group). 2012 mice were transplanted with $3X10^5$ Ly5.2 cells/mouse (4 mice). Weeks are the times after transplant that the mice were analyzed. (n), number of mice surviving/group; ND, not done. Data are presented ±SEM.

EXAMPLE 4: Hematopoietic activity of a stromal cell transmembrane protein containing epidermal growth factor-like repeat motifs

Primitive hematopoietic stem cells are closely associated with discrete *in vivo* microenvironments. These "niches" are thought to provide the molecular signals that mediate stem cell differentiation and self renewal. The fetal liver microenvironment was dissected into distinct cellular components by establishing an extensive panel of stromal cell lines. One particular cell line maintains repopulating stem cells for prolonged *in vitro* culture periods. A subtraction cloning strategy has yielded a cDNA which encodes a cell surface glycoprotein with a restricted pattern of expression among stromal cell lines. This

molecule, previously identified as delta-like/pre-adipocyte factor-1, contains epidermal growth factor-like repeats which are related to those in the notch/delta/serrate family of proteins. The potential role of this molecule in hematopoietic stem/progenitor cell regulation was investigated. The delta-like protein displays activity on purified stem cells by promoting the formation of "cobblestone areas" of proliferation. These cobblestone areas contain both primitive high-proliferative potential progenitors and *in vivo* repopulating stem cells.

Introduction

The positive and negative regulatory mechanisms that govern the proliferation, self-renewal and differentiation of primitive hematopoietic stem cells are complex and poorly understood (Ogawa, M. (1993) *Blood* **81**, 2844-2853). Numerous cytokines have been identified that, when used *in vitro* appear to act directly on purified stem cells by promoting proliferation and differentiation. However, attempts to demonstrate the *in vitro* maintenance and/or expansion of transplantable pluripotent stem cells using defined cytokine combinations have been largely unsuccessful (Knobel, K. M., et al., 1994; Peters, S. O., et al., 1995). Moreover, it is unclear to what extent any currently identified cytokines reflect mechanisms that are responsible for regulating normal, *in vivo*, stem cell behaviors. It is widely accepted that *in vivo*, stem cells are intimately associated with discrete microenvironmental "niches" (Wolf, N. S. 1979). Such niches are likely sources for the molecular signals which collectively mediate the differentiation and self-renewal of stem cells. Indeed, it has long been possible to demonstrate that preestablished stromal cell monolayers derived from hematopoietic tissues can support long-term hematopoiesis *in vitro* (Dexter, T. M., Allen, T. D. & Lajtha, L. G. 1977). The long-term nature of these cultures, together with the continuous production of committed progenitor cells suggest that both self-renewal and commitment decisions can occur *in vitro*. At the cellular level, the hematopoietic microenvironment consists of numerous distinct cell types. Previous studies have shown that this cellular heterogeneity reflects a similarly broad heterogeneity in terms of hematopoietic supportive abilities (Deryugina, E. I., et al., 1994). Some cloned stromal cell lines can support stem cell activity *in vitro*, while others are ineffective. Similarly, distinct stromal cell types appear to influence the outcomes of stem cell differentiation processes (Friedrich, C., et al., 1996). Recent studies have shown that stromal cell lines that efficiently maintain long-term transplantable stem cells *in vitro* for prolonged intervals represent a small fraction of the total stromal cell population (Wineman, J., et al., 1996). A fetal liver stromal cell line, AFT024 was identified which maintains high levels of

transplantable multilineage stem cell activity for extended *in vitro* culture periods (Moore, K. A., Ema, H. & Lemischka, I. R. (1997) (See, Example 3). The stem cells used to initiate these cultures are highly purified. It is, therefore, likely that in this system the mechanisms that mediate stem cell maintenance do so by acting directly on primitive stem cells. A different fetal liver-derived cell line, 2018, fails to maintain long-term repopulating stem cell activity. A PCR-based RNA expression analysis of 13 cytokines reveals qualitatively identical expression patterns in AFT024 and 2018. It wastherefore, hypothesized that the hematopoietic supportive ability of AFT024 is, at least in part, mediated by novel gene products not expressed in 2018.

Using a subtractive hybridization approach a number of cDNA clones were identified which were specifically expressed in AFT024 but not in 2018. The sequence of one cDNA was identical to a molecule that encodes a transmembrane protein that contains six epidermal growth factor (EGF) repeat motifs. The EGF-like repeat sequences of this molecule, variously known as delta-like (dlk) (Laborda, J., et al., 1993), preadipocyte factor-1 (Smas, C. M. & Sul, H. S, 1993), and stromal cell protein-1 (Genbank, D16847), are most closely related to those present in the notch/delta/serrate family of signaling proteins. In *Drosophila* and *Caenorhabditis*, these molecules are required for correct cell-fate specification decisions in a variety of tissues (Rebay, I., et al., 1991). Vertebrate homologs of the notch/delta/serrate family have been identified (Ellisen, L. W., et al., 1991; Bettenhausen, B., et al., 1995; Lindsell, C. E., et al.,1995). While the exact functional relationship of dlk to the activities of this family of molecules is unclear, in one *in vitro* study, it has been shown to block adipocyte differentiation (Smas, C. M. & Sul, H. S, 1993). No studies have been reported that demonstrate a hematopoietic function for dlk. Expression analyses and these observations show a limited temporal pattern of dlk expression during murine fetal development which coincides with the time period of hematopoietic stem cell expansion (Smas, C. M. & Sul, H. S, 1993).

Functional studies were undertaken to determine if dlk can act as a hematopoietic regulator. This molecule affects highly enriched stem cell populations by promoting "cobblestone area" (CSA) colony formation in dexter-type stromal cocultures. These CSA colonies contain an expanded population of primitive, high proliferative potential myeloid-erythroid progenitors. These cultures also contain stem cells capable of *in vivo* engraftment at levels equivalent to those present in parallel AFT024 supported cultures. It is proposed that dlk represents one molecular component responsible for the hematopoietic supportive

ability of AFT024. As such, dlk may define a novel molecular pathway of stem cell regulation by the microenvironment.

Materials and Methods

Cell Lines and Culture. The fetal liver stromal cell lines used in this study were derived as previously described (Wineman, J., et al., 1996). Cells were routinely cultured in DMEM containing 10% fetal bovine serum (FBS) and 50 uM b-mercaptoethanol (2-ME), and maintained at 31^0-33^0C, 5% CO_2. For long-term cocultures with hematopoietic stem cells, confluent monolayers were irradiated (20 Gy), placed in modified Dexter media (DMEM, 10% FBS, 10% horse serum, 50 uM 2-ME, 0.1 uM hydrocortisone) and maintained at 37^0 C, 5% CO_2 with weekly media changes. NIH3T3 cells were obtained from ATCC.

dlk Expression Analysis. Total RNAs from stromal cell lines were poly A^+ selected, Northern blotted, and hybridized to ^{32}P-labeled probes according to standard protocols (Sambrook, J., Fritsch, E. F. & Maniatis, T, 1989). A 600 bp dlk-cDNA clone from the AFT024 subtracted library was used as a probe. cDNA templates for RT-PCR were prepared according to manufacturers' protocols (GIBCO/BRL). Oligonucleotide primers were: sense 5'- GACCCAGGCTGCCCC-3' (SEQ.ID.No.:85) and antisense 5'-GGTACTCTTGTTGAG-3' (SEQ.ID.No.:86). For analysis of dlk expression at the protein level, antisera specific for dlk was generated by immunizing rabbits with a Flag-dlk fusion protein (described below). Resultant antibodies were purified by affinity chromatography. Cell surface expression of dlk in stromal cell lines was accomplished by flow cytometry. Cells were incubated with dlk antibody and a similarly prepared irrelevant control antibody. Specific labeling was developed by donkey anti-rabbit-fluorescein isothiocyanate (Jackson ImmunoResearch). Stained cells were analyzed on a Becton Dickinson FACScan using Cell Quest software.

dlk Fusion Protein Preparation. The expression plasmid pCD4-Ig contains cDNA for the extracellular domain of human CD4 fused to genomic sequences of the human immunoglobin heavy chain (Zettlmeissl, G., et al., (1990). cDNA for CH2-CH3 of human IgG_1 (Goodwin, R. G., et al., 1990) was cloned into *Eco*RI and *Not*I sites of pcDNA3 (Invitrogen) to give the plasmid KB52.3.2. cDNA encoding the extracellular domain of dlk was obtained by RT-PCR with primers BP 151 and BP 152 using total RNA from NIH 3T3 cells as template. The resulting PCR fragment was cloned into KB52.3.2 via *Hind*III and

*Eco*RI sites to obtain the soluble dlk-Ig expression plasmid. pdlk-Ig or pCD4-Ig were transfected into NIH3T3 cells together with pSVNeo and stable clones were isolated. Soluble CD4-Ig and dlk-Ig fusion proteins were harvested and then purified by affinity chromatography on HiTrap Protein G-sepharose (Pharmacia). Primers: sense BP 151, 5'GAGGGTACCAAGCTTCGTGGTCCGCAACCAGAAG-3' (SEQ.ID.No.: 87); anti-sense BP 152, 5'-CTCAGATCTGAATTCGGCCTGTCCCTCGGTGAGGAG-3' (SEQ.ID.No.: 88).

Flag-dlk fusion protein was used to immunize rabbits for the production of dlk antiserum. The protein expression plasmid pcDNA3-Flag is a modification of the plasmid pcDNA3 (Invitrogen) and contains the coding region for the Flag peptide (DYKDDDDKI) (Hopp, T. P., et al., 1988) as well as a *Bgl*II restriction site. A cDNA fragment encoding the extracellular domain of dlk was obtained by RT-PCR using RNA from NIH3T3 cells. Primers: sense BP 155, 5'-GACAAGATCTCAGCTGAATAGCGACCCACCCTGTG-3' (SEQ.ID.No.: 89); antisense BP 154, 5'-GCATCTAGAGCGGCCGCTCAGGCCTGTCCCTCGGTGAGGAG-3' (SEQ.ID.No.: 90). The PCR fragment was ligated into pcDNA3-Flag to yield pFlag-dlk. pFlag-dlk was transfected into cos cells. Purification of the Flag-dlk protein from cos-conditioned media was performed according to manufacturer's directions using the Flag monoclonal antibody, M1, immobilized on agarose (International Biotechnologies).

Plasmid Constructs and Stable Transfection. Full-length murine dlk cDNA was obtained by RT-PCR with primers BP 151 (see above) and antisense BP 200: 5'GCATCTAGAGCGGCCGCGAACGCTGCTTAGATCTCCT-3' (SEQ.ID.No.:91), using total RNA from NIH3T3 cells as template. The product was subcloned into the vector pCRII (Invitrogen) and then cloned into a retroviral expression vector (Kitamura, T., et al., 1995), (G. Nolan, Stanford University), via the primer-encoded *Hind*III and *Not*I sites. Supercoiled plasmid was transfected into BFC012 stromal cells together with the pZeo (Invitrogen) selectable marker and selected in 50 ug/ml Zeocin (Invitrogen). BFC012 cells also were transfected with pZeo alone and selected as above. Clones from both selected populations were isolated and all remaining colonies (100-200 per dish) were pooled and expanded as populations.

Hematopoietic Stem Cells and *In Vitro* Hematopoietic Assays. Hematopoietic stem cell populations were derived from wild type, Ly5.2-C57Bl/6J (Jackson Laboratories), day

14 fetal liver, enriched for the AA4.1$^+$, Sca-1$^+$, c-*kit*$^+$, and lin$^{lo/-}$ phenotype, by immunopanning and fluorescence-activated cell sorting as described (Jordan, C. T., et al., 1995). Adult bone marrow (BM) was used directly after density centrifugation and immunomagnetic bead depletion or was further enriched for Sca-1$^+$, c-*kit*$^+$, lin$^{lo/-}$ cells by flow cytometry as described (Okada, S., et al., 1992). Cell sorting and data analysis was accomplished with a Becton Dickinson FACS Vantage using Cell Quest software. Stromal cell/stem cell cocultures were initiated in 12-well trays with 300-1,000 enriched stem cells per well. Cobblestone areas were quantitated by inverted-phase microscopy as described (Ploemacher, R. E., et al. , 1991). Clonogenic progenitor assays were performed with either freshly purified stem cells or cells harvested from the stromal cocultures. These were cultured in cytokine-containing semisolid media according to the manufacturer's recommendations (Stem Cell Technologies, Vancouver, BC). Soluble dlk and control fusion proteins were added to semisolid progenitor assays at concentrations of 0.1, 0.5 and 1.0 ug/ml and also to BFC012 stromal cocultures at concentrations of 0.1 ug/ml. Fusion protein was replenished weekly in the stromal cocultures.

Competitive repopulating transplantation assay. Cultured cells were harvested, combined with fresh unfractionated BM obtained from congenic C57Bl/6 Ly5.1 mice (National Cancer Institute) and transplanted into lethally irradiated (10 Gy, split dose 3 h apart from a ^{137}Cs source, 1 Gy/min) Ly5.1 recipient mice. Each mouse received 2 X 10^5 competitor BM cells and a fraction of the cocultured stem cells. Mice were bled by capillary puncture of the orbital venous plexus and 100 ul was collected; red blood cells were removed by NH_4Cl lysis. The nucleated cells were stained for the Ly5.2 (CD45.2) allelic marker using either fluorescein isothiocyanate-labeled directly conjugated Ly5.2 monoclonal antibody or a biotinylated form developed with streptavidin conjugated to Texas red. Cells also were stained with directly conjugated antibodies to lineage markers. All antibodies and chromogens were obtained from Pharmingen. Flow cytometric analysis was done on a Becton Dickinson FACS Vantage using Cell Quest software.

Results

Genes expressed in AFT024 but not in 2018 were identified by a subtractive cloning approach. Sequence analysis identified one of these AFT024-specific clones as dlk. Expression studies, (Figure 19A) show high levels of dlk in AFT024 and subclones isolated from this line, but undetectable levels in 2018 and BFC012. The latter two stromal cell lines

do not support repopulating stem cells. The PA6 stromal cell line and NIH Swiss 3T3 cells both show expression of dlk and were the cell sources for identification of SCP-1 and dlk, respectively. PA6 cells have been shown to support *in vitro* hematopoiesis and long-term, *in vivo* repopulating stem cells (Kodama, H., et al., 1992). Swiss 3T3 cells are also capable of supporting multipotent hematopoietic stem cells *in vitro*, promoting CSAs and maintaining *in vivo* spleen colony-forming units (CFU-S) (Roberts, R. A., et al., 1987). Interestingly, an additional fetal liver stromal cell line, 2012, which has some degree of stem cell supporting activity (Wineman, J., et al., 1996), and its subclones also express dlk. Furthermore, an RT-PCR analysis (40 cycles) of an additional 10 fetal liver-derived stromal cell lines and several other lines, shows detectable levels of dlk in only two additional lines (Figure 19B). These two cell lines (CFC032 and CFC008) can maintain some level of long-term transplantable stem cell activity present in whole BM (Wineman, J., et al., 1996). A correlation between a stromal cell line's ability to support stem cells and the expression of dlk was suggested. Therefore, functional studies were undertaken in order to delineate if dlk can act on or modulate hematopoietic stem cells.

Soluble dlk protein was added to progenitor cultures in semi-solid media. The soluble protein consisted of the dlk extracellular domain fused to the Fc portion of human IgG_1. The stem cell sources in these assays were highly enriched fetal liver cells ($AA4.1^+$, $lin^{lo/-}$, $Sca\text{-}1^+$, c-*kit* $^+$). The influence of soluble dlk on hematopoietic progenitor colony-formation was assessed. As shown in Table 6, no differences were noted either in the number, sizes, or lineage compositions of colonies. Identical results were obtained at dlk concentrations ranging from 0.1 to 1.0 ug/ml. In addition, no differences were noted in similar studies using enriched BM cells ($Sca\text{-}1^+$, c-*kit*$^+$, $lin^{-/lo}$).

Evidence for a positive effect of the dlk protein on stem/progenitor cells was observed when the soluble form was added to dexter-type cocultures. For these studies a stromal cell line (BFC012) was used that neither expresses endogenous dlk (see Figure 19) nor maintains significant *in vitro* hematopoiesis. In four experiments, two each using highly enriched adult BM and fetal liver stem cells, we monitored the appearance of CSAs over time. These colonies provide a convenient, quantitative estimate of hematopoietic activity initiated by primitive stromal dependent stem/progenitor cells. As shown in Figure 20, the addition of soluble dlk (0.1 ug/mL) results in an approximately 2-fold increase in the number of CSAs initiated by purified fetal liver or BM stem cells over a 2 week time period ($P= 0.001$ for dlk vs control and $P= 0.01$ for dlk vs no additive, Student's t-test). There was no

difference in the numbers of CSA observed in BFC012/stem cell cultures with or without control fusion protein (mean of no additive/control = 0.96±0.11)

In order to assess the activity of the normal transmembrane form of dlk, a full-length dlk cDNA was transfected into BFC012 cells. Expression of the introduced dlk was demonstrated at the RNA (Northern blot) and protein levels using both Western blot and flow cytometric analyses with rabbit anti-dlk antibodies. The flow cytometry data are presented in Figure 21.

Initially, dlk-expressing transfected populations (BFC-dlk) were compared to a negative control "mock" transfected population of BFC012 cells. A 4- to 6-fold increase in the number of CSAs was observed in two separate experiments. The maintenance of CSAs was transient, lasting less than 2 weeks. No further hematopoietic activity was observed during an additional 2 weeks of culture. Once dlk-expressing clones had been identified from the transfected populations, they were studied for their ability to support CSAs in experiments designed to more precisely identify the time course of hematopoietic activity. Ficoll-separated, lineage depleted BM was used in these experiments. Five different negative control, non-dlk expressing BFC012 cell groups (parental BFC012 cells, two "mock" transfected populations, and two "mock" transfected clones) and 3 dlk-expressing BFC012 cell groups (one transfected population and two clones) were studied. The data are presented in Figure 22A. Neither the negative control BFC populations nor the "mock" transfected BFC clones supported high numbers of CSAs. In contrast, the BFC-dlk populations and the two individual dlk-expressing clones supported significantly greater numbers of CSAs at all time points studied ($P < 0.001$ days 3,4, and 5; $P < 0.01$ days 6 and 7, Student's t-test). As observed previously, all the CSAs were transient. This experiment also indicated that the dlk-promoted hematopoietic activity peaks early, at 4 days, in this culture system. Three additional experiments using purified (AA4.1$^+$, lin$^{lo/-}$, Sca-1$^+$, c-*kit*$^+$) fetal liver stem cells were performed using two individual clones, BFC-dlk-5 and a "mock" transfected negative control BFC-Zeo-1. The results are presented in Figure 22B. There was a dramatic and significant difference in the number of CSAs observed in the BFC-dlk-5 cultures compared to the control line ($P < 0.001$, days 4, 6, and 8, Student's t-test). As before, the effect was transient and the CSA declined in number over 2 weeks. AFT024 was included as a positive control and, in each of the three experiments, verified the quality of the input purified stem cells. In the first week of culture the numbers of CSAs observed on AFT024 were similar to the numbers in the BFC-dlk5 cultures.

In order to address the "primitiveness" of the cells that give rise to the CSA observed in the BFC-dlk cocultures, a series of *in vitro* replating experiments were performed. Individual wells were harvested at various time points of coculture and the cells were plated into semisolid cytokine-containing media. The numbers and lineage compositions of the colonies were scored after 8-12 days. As shown in Figure 23A, the CSAs obtained from day 4 BFC-dlk-5 cocultures contained numerous progenitors capable of extensive proliferation and multilineage differentiation. The total number of progenitors from the dlk-expressing cultures at day 4 was significantly expanded compared to the content in the freshly purified uncultured stem cell population (P= 0.01, Student's t-test). The number and lineage composition of colonies derived from parallel day 4 AFT024 cultures was nearly identical to BFC-dlk-5 derived colonies. The content of CSAs replated at day 6 from the BFC-dlk-5 cocultures was devoid of multilineage colonies, although CFU-granulocyte-macrophages were maintained at high levels; the progenitor content in the BFC-dlk-5 cocultures continued to decrease when next sampled at 10 days. In contrast, few progenitors could be demonstrated in the BFC-Zeo-1 cultures (P= 0.001, BFC-dlk-5 vs. BFC-Zeo-1, Student's t-test) (Figure 23A). Taken together, the data strongly suggest that dlk acts to promote stromal-dependent colony-formation by primitive cells capable of yielding large numbers of committed progenitors, including those endowed with a high proliferative capacity and multilineage differentiation potential. The lack of CSAs and significant progenitor maintenance in the BFC-Zeo-1 cultures argues that expression of dlk in the transfected BFC012 cells is responsible for both their ability to support CSAs and to generate/maintain primitive progenitors.

In order to determine if the CSA-containing cultures supported by BFC-dlk-5 contained stem cells capable of *in vivo* engraftment, portions of the same day 4 cocultures that were plated into progenitor assays also were used to transplant mice in competitive repopulation assays. Shown in Figure 23B are the results from two independent experiments analyzed at 10 weeks after transplant. The same BFC-dlk-5 cultures that contain CSAs and primitive CFU-high proliferativ potential (HPP)-Mix progenitors also contain repopulating stem cells at levels equal to those maintained in parallel AFT024 cocultures. In addition, a significant difference exists in the levels of repopulating stem cells derived from dlk-expressing cocultures compared to non-dlk expressing BFC012 cells (P= 0.05, Student's t-test). Multi-color flow cytometric analyses also demonstrated that both myeloid and lymphoid Ly5.2 cells are present in these animals. A subsequent analysis of these animals at

22 weeks demonstrated lower levels of repopulation with Ly5.2 cells derived from the AFT024 and BFC-dlk-5 supported cultures (data not shown). Most significantly, no repopulation was observed at any time point in mice that received cells cocultured on the non-dlk-expressing monolayers (Figure 23B).

Discussion

As part of an ongoing effort to understand the biology of the hematopoietic microenvironment, a panel of stromal cell lines from midgestation fetal liver was established and characterized. Among these cell lines, the AFT024 line has the ability to maintain nearly quantitative levels of transplantable stem cell activity for extended *in vitro* time periods (Moore, K. A., Ema, H. & Lemischka, I. R.,1997). Because these cultures are initiated with highly purified stem cell populations it is likely that the AFT024-derived molecular mechanisms responsible for this ability act directly on the stem cell population. Other stromal lines that fail to maintain stem cell activity were also identified. These observations facilitated a subtractive hybridization approach aimed at identifying potential candidate molecules whose collective actions may be responsible for the AFT024 stem cell maintenance activity. This effort has identified dlk, a transmembrane molecule containing six EGF-like repeat motifs. Although lacking the DSL motif indicative of the notch ligands delta and serrate (Tax, F. E., Yeargers, J. J. & Thomas, J. H., 1994), dlk is most closely homologous to delta/notch/serrate when compared to other EGF-like repeat containing molecules (Laborda, J., et al., 1993; Smas, C. M. & Sul, H. S., 1993). The predominant role of these types of molecules in cell growth and differentiation led us to investigate the potential role of dlk in hematopoiesis. Constitutive expression of translocated human notch (Tan-1) is found in a T-cell leukemia (Ellisen, L. W., 1991). Moreover the expression of Tan-1 in primitive human stem cells has been demonstrated (Milner, L. A., et al., 1994). Nevertheless, a functional role in hematopoiesis for the notch ligands Jagged (Lindsell, C. E., 1995) and Delta-like-1 (Bettenhausen, B., et al., 1995) has not been described. dlk expression is highly restricted in a panel of stromal cell lines. Two lines, AFT024 and 2012, which maintain repopulating stem cell activity *in vitro*, express dlk, whereas two non-supportive cell lines, 2018 and BFC012, do not. Interestingly, the S17 stromal cell line which is considered to be a potent stem cell supporter (Wineman, J. P., et al., 1993) does not express detectable levels of dlk. The S17 cell line was derived from adult BM (Collins, L. S. & Dorshkind, K., 1987)). The other lines described are all derived from fetal sources (AFT024, 2012, and NIH 3T3 cells) or from newborn calvaria (PA6 cells). It is therefore

possible that dlk acts in a developmentally regulated fashion. An extensive analysis of dlk expression in adult BM stroma is currently underway. Taken together, the data suggest that, at least in fetal stromal cell types, there exists a correlation between hematopoietic supporting ability and the expression of dlk.

The potential activity of both soluble and transmembrane dlk protein, on highly purified stem cell populations were directly measured using *in vitro* and *in vivo* assays. Initial experiments designed to ask if dlk can enhance colony formation in cytokine-rich semisolid assay systems were negative. These results may indicate that: (i) progenitor cells capable of colony formation in semisolid assays do not respond to dlk, (ii) the collection of cytokines present in the semisolid cultures may "mask" an effect(s) of added dlk or (iii) that the soluble form of dlk requires a stromal monolayer to mediate its effects. The first possibility can be addressed more extensively in delta-type assays, where stem/progenitor cells are first cultured in suspension in serum-free media containing various cytokine combinations, with and without dlk, and then replated into colony assays (Muench, J. O., Firpo, M. T. & Moore, M. A., 1993). The second possibility can be addressed by more extensive studies using subsets of the cytokines present in our initial studies. These experiments are underway. As a first step to address the third possibility, we added soluble dlk to preestablished BFC012 monolayers. Using both purified BM and fetal liver stem cell populations, a significant increase in CSA colony formation was observed in the dlk supplemented cultures (Figure 20). This was a surprising result, given that dlk is a transmembrane protein; however, before its cDNA cloning, a soluble form of dlk was identified as FA1 or fetal antigen 1 (Jensen, C. H., et al., 1994). A role in hematopoiesis was not indicated in these studies, but expression was detected in stroma of placental villi, in yolk sac blood islands and in fetal liver (Jensen, C. H., et al., 1994). It is of interest to determine if a soluble form is produced by the stromal cell lines that express dlk. An additional explanation, for the effects observed with the soluble form added to stromal/stem cell cocultures, is that they may be facilitated by the Fc portion of the fusion protein. It is possible that Fc receptors expressed by some of the hematopoietic cells in the cultures are able to sequester and present the dlk-Fc fusion protein more effectively. This possibility can be addressed by using a different type of soluble dlk protein. These studies have been initiated. Alternatively, the soluble dlk may be sequestered and thus presented by the stromal cell extracellular matrix. In order to further address the third possibility, an intact transmembrane form of dlk was introduced into the BFC012 stromal cell line. Initially, dlk

transfected BFC012 cell populations were compared to BFC012 cells transfected with the selectable marker alone. In these studies, the dlk expressing monolayers were more effective at promoting CSA colonies. As with the soluble dlk experiments, the CSAs appeared early and were transient. When cells were replated from the BFC-dlk supported cocultures onto an AFT024 monolayer a reiteration of the burst of CSAs was seen that was maintained for 3 weeks. In addition, replating of these cocultures revealed a high progenitor content (~1 in 10) that included a high percentage (43%) of multipotential colonies including HPP-Mix. In the BFC-Zeo control populations neither replatable CSA nor CFU progenitors were maintained. Further experiments with individual clones from the transfected populations confirmed and extended the results obtained with the populations, demonstrating highly significant differences in the numbers of developing CSAs (Figure 22). However, in two experiments we observed that one dlk-transfected BFC012 clone, which expresses a very high level of dlk, supported fewer CSAs than non-dlk-expressing control cells. These cultures also suggested differentiation phenomena, as indicated by the number of rapidly accumulating nonadherent cells. Experiments utilizing this cell line were not included in our analyses. It is possible that there may be a threshold level of dlk expression necessary in these cultures and when it is surpassed the cells differentiate and die rapidly in the culture mileau provided by BFC012 cells. In addition, it is possible that an aberrant form of the dlk protein is made by this line. Further studies are necessary to clarify this issue. Nevertheless, in Figure 22A, dlk-transfected BFC012 cells (one population and two clones) show a significant enhancement of CSA formation compared to controls.

The observed low level maintenance of competitive repopulating stem cells in short-term dlk-expressing cocultures is of interest even though the activity diminished over time. These studies show that the ectopic expression of a single molecule (dlk) in a previously nonsupporting stromal cell line restores or enables hematopoietic support. This is demonstrated by maintenance of three different stem/progenitor cell compartments: (i) CFU-HPP-Mix, (ii) CSA, and (iii) short-term *in vivo* repopulating stem cells. It is also of interest that both qualitatively and quantitatively similar stem/progenitor cell compartments are maintained in short-term AFT024 supported cocultures (Moore, K. A., Ema, H. & Lemischka, I. R., 1997).

Two mechanisms underlying the effects of dlk are considered. First, it may be that some level of dlk expression is sufficient to retard potent differentiation signals provided by the BFC012 cell line. Second, dlk may provide a proliferative stimulus not normally

produced by BFC012. A more direct and perhaps relevant assay will be to eliminate the expression of dlk in AFT024 cocultures, thus maintaining other components that make up the culture "milieu." Studies to evaluate potential neutralizing antibodies and various dlk antisense strategies are underway.

The failure of BFC-dlk-5 supported CSAs to persist for periods longer than 1 to 2 weeks also may suggest the existence of other molecules in AFT024 that facilitate hematopoiesis. In this regard, it is interesting that our subtraction screen has yielded several other clones with expression patterns very similar to dlk. Eventually, with the addition of dlk and other AFT024 specific molecules it may be possible to reconstruct a supportive phenotype. This should lead towards a further understanding of the *in vivo* hematopoietic microenvironment. In summary, it is proposed that dlk represents one molecular component responsible for the hematopoietic supportive activities of the AFT024 cell line. As such, dlk may define a novel molecular pathway of stem cell regulation by the hematopoietic microenvironment.

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This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid derived from an isolated hematopoietic stem cell, the isolated nucleic acid comprising the following characteristics:
   - specifically expressed in the hematopoietic stem cell; and
   - encoding a hematopoietic stem cell – specific protein.

2. An isolated nucleic acid of claim 1, the isolated nucleic acid further comprising the following characteristic:
   - capable of hybridizing under standard conditions with a sequence selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof.

3. The isolated nucleic acid of claim 1, the isolated nucleic acid further comprising the characteristic of encoding a protein capable of modulating hematopoietic stem cell activity.

4. The isolated nucleic acid of claim 3, wherein the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication.

5. The isolated nucleic acid of claim 3, wherein the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor

6. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the nucleotide sequence of SEQ.ID.No.: 72, an analog thereof, or a portion thereof.

7. The isolated nucleic acid of claim 1, wherein the hematopoietic stem cell is a primitive hematopoietic stem cell.

8. The isolated nucleic acid of claim 7, wherein the primitive hematopoietic stem cell is selected from the group consisting of an umbilical cord cell , a bone marrow cell and a fetal liver cell.

9. The isolated nucleic acid of claim 7, wherein the primitive hematopoietic stem cell is selected from the group consisting of a AFT024 cell, a 2012 cell and a 2018 cell.

10. A composition comprising the nucleic acid of claim 1, wherein the nucleic acid comprises one selected from the group consisting of SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39, SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a portion thereof.

11. The nucleic acid of claim 1, wherein the nucleic acid is selected from the group consisting of DNA, RNA and cDNA.

12. A vector comprising the nucleic acid of claim 1.

13. The vector of claim 12, wherein the vector comprises viral or plasmid DNA.

14. An expression vector comprising the nucleic acid of claim 1 and a regulatory element.

15. A host vector system which comprises the expression vector of claim 12 in a suitable host.

16. The host vector system of claim 15, wherein the suitable host is selected from the group consisting of a bacterial cell, a eukaryotic cell, a mammalian cell and an insect cell.

17. An isolated hematopoietic stem cell specific protein or a portion thereof encoded by the nucleic acid of claim 1.

18. The isolated hematopoietic stem cell of claim 16, further comprising the following characteristic:

- capable of modulating hematopoietic stem cell activity.

19. The isolated hematopoietic stem cell of claim 18, wherein the activity is selected from the group consisting of hematopoietic stem cell differentiation and hematopoietic stem cell replication.

20. The protein of claim 17, wherein the protein is selected from the group consisting of a growth factor, a transcription factor, a splicing factor, a capping factor, a transport protein, a translation factor, and a replication factor.

21. The protein of claim 17, the protein having substantially the same amino acid sequence as one selected from the group consisting of SEQ.ID.No.: 42, SEQ.ID.No.:

44, SEQ.ID.No.: 46, SEQ.ID.No.: 48, SEQ.ID.No.: 50, SEQ.ID.No.: 52, SEQ.ID.No.: 54, SEQ.ID.No.: 56, SEQ.ID.No.: 58, SEQ.ID.No.: 60, SEQ.ID.No.: 62, SEQ.ID.No.: 64, SEQ.ID.No.: 66, SEQ.ID.No.: 68, SEQ.ID.No.: 70, SEQ.ID.No.: 71, and SEQ.ID.No.: 73.

22. A nucleic acid probe capable of specifically hybridizing with the nucleic acid of claim 1 under standard conditions.

23. An antibody capable of specifically binding to the protein of claim 17 without substantially cross-reacting with a non-stem cell specific protein or homologs thereof under conditions permissive to antibody binding.

24. A cell capable of producing the antibody of claim 23.

25. A method for identifying the presence of a primitive hemopoietic stem cell in a sample comprising:

(a) contacting the sample with the antibody of claim 23 under conditions permissive to the formation of an antibody complex; and

(b) detecting the presence of the complex formed in step (a), the presence of a complex formed indicating the presence of a primitive hemopoietic stem cell in the sample.

26. The method of claim 25, wherein the antibody is labeled with a detectable marker.

27. The method of claim 26, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, magnetic bead, dye, flourescent marker and biotin.

28. A method for generating a stem cell/progenitor cell from a primitive hematopoietic cell in a sample comprising contacting the sample with the protein of claim 17.

29. A method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity comprising:

(a) contacting the hematopoietic stem cell with the sample;

(b) determining the hematopoietic stem cell activity;

(c) comparing the hematopoietic stem cell activity determined in step (b) with the activity determined in the absence of the compound an increase or decrease in hematopoietic stem cell activity indicating the presence in the sample of a compound that modulates hematopoietic stem cell activity.

30. The method of claim 29, wherein the activity is selected from the group consisting of gene expression, replication, differentiation, transplantation, and self regeneration.

31. A compound identified by the method of claim 29, previously unknown.

32. A method for identifying primitive hematopoietic stem cell-specific nucleic acids , comprising:

(a) creating a primitive hematopoietic stem cell cDNA library and a non-primitive stem cell immune cell cDNA library; and

(b) subtracting the two libraries, thereby identifying primitive stem cell specific nucleic acids.

33. The method of claim 32, step (b) comprising:

(i) contacting the nucleic acids of the stem cell and non-stem cell libraries with each other under conditions permissive to hybridization, thereby forming hybrid complexes;

(ii) separating the hybrid complexes formed in step (b) from the nucleic acids which did not form complexes;

(iii) isolating the nucleic acids which did not form complexes, thereby identifying hematopoietic stem cell specific nucleic acids.

34. The method of claim 33, step (ii) further comprising amplification of the nucleic acids.

35. The method of claim 33, step (iii) further comprising ampliciation of the nucleic acids which did not form complexes.

36. The method of claim 33, further comprising displaying the amplified DNA on a chromatography gel.

37. The method of claim 32, step (b) comprising differential display of the two libraries, thereby identifying primitive stem cell specific nucleic acids.

38. The method of claim 32, step (b) comprising representation difference analysis of the two libraries, thereby identifying primitive stem cell specific nucleic acids.

39. The method of claim 32 further comprising cloning the stem cell specific nucleic acid.

40. The method of claim 32, wherein the stem cell is selected from the group consisting of AF024, 2012, and 2018.

41. A nucleic acid identified by the method of claim 32

42. A composition comprising the compound of claim 31 and a carrier.

43. A pharmaceutical composition comprising the compound of claim 31 and a pharmaceutically acceptable carrier.

44. A primitive hematopoietic stem cell specifically expressing one selected from the group consisting of: SEQ.ID.No.: 1, SEQ.ID.No.: 2, SEQ.ID.No.: 3, SEQ.ID.No.: 4, SEQ.ID.No.: 5, SEQ.ID.No.: 6, SEQ.ID.No.: 7, SEQ.ID.No.: 8, SEQ.ID.No.: 9, SEQ.ID.No.: 10, SEQ.ID.No.: 11, SEQ.ID.No.: 12, SEQ.ID.No.: 13, SEQ.ID.No.: 14, SEQ.ID.No.: 15, SEQ.ID.No.: 16, SEQ.ID.No.: 17, SEQ.ID.No.: 18, SEQ.ID.No.: 19, SEQ.ID.No.: 20, SEQ.ID.No.: 21, SEQ.ID.No.: 22, SEQ.ID.No.: 23, SEQ.ID.No.: 24, SEQ.ID.No.: 25, SEQ.ID.No.: 26, SEQ.ID.No.: 27, SEQ.ID.No.: 28, SEQ.ID.No.: 29, SEQ.ID.No.: 30, SEQ.ID.No.: 31, SEQ.ID.No.: 32, SEQ.ID.No.: 33, SEQ.ID.No.: 34, SEQ.ID.No.: 35, SEQ.ID.No.: 36, SEQ.ID.No.: 37, SEQ.ID.No.: 38, SEQ.ID.No.: 39,

SEQ.ID.No.: 40, SEQ.ID.No.: 41, SEQ.ID.No.: 42, and SEQ.ID.No.: 43, SEQ.ID.No.: 45, SEQ.ID.No.: 47, SEQ.ID.No.: 49, SEQ.ID.No.: 51, SEQ.ID.No.: 53, SEQ.ID.No.: 55, SEQ.ID.No.: 57, SEQ.ID.No.: 59, SEQ.ID.No.: 61, SEQ.ID.No.: 63, SEQ.ID.No.: 65, SEQ.ID.No.: 67, SEQ.ID.No.: 72 or a functional portion thereof.

45. A primitive hematopoietic stem cell specifically expressing a nucleic acid identified by the method of claim 32.

46. A method for treating a condition in a subject comprising administering to the subject a therapeutically effective amount of the composition of claim 43.

47. The method of claim 46 wherein the condition is an immune system condition.

48. The method of claim 46 wherein the condition is leukemia.

49. A method of introducing an exogenous nucleic acid into a hematopoietic stem cell comprising contacting the stem cell with the composition of claim 42.

50. A method of ex vivo expansion of hematopoietic stem cells comprising contacting the cell with the composition of claim 42.

FIGURE 1

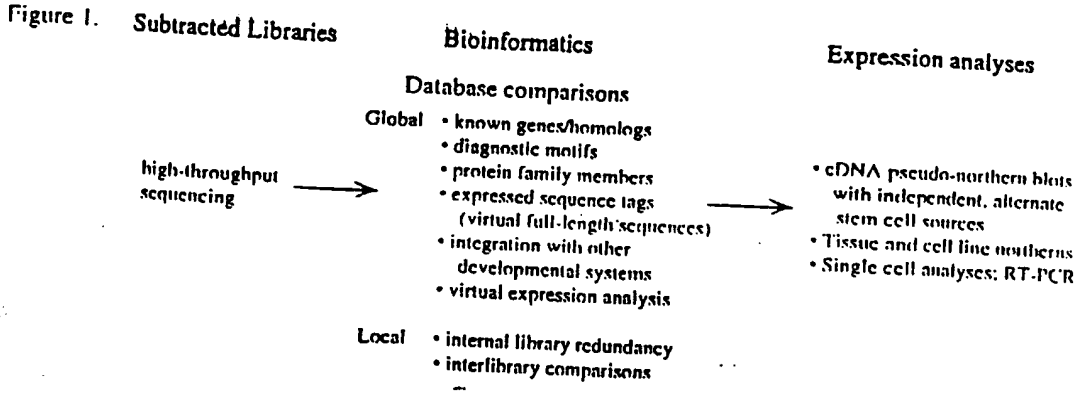


Figure 1.
Subtracted Libraries
Bioinformatics
Expression analyses
Database comparisons
Global
• known genes/homologs
• diagnostic motifs
• protein family members
• expressed sequence tags (virtual full-length sequences)
• integration with other developmental systems
• virtual expression analysis
high-throughput sequencing
• cDNA pseudo-northern blots with independent, alternate stem cell sources
• Tissue and cell line northerns
• Single cell analyses: RT-PCR
Local
• internal library redundancy
• interlibrary comparisons

FIGURE 2

Figure 2.

Cyt-19 SA61 Smc-34

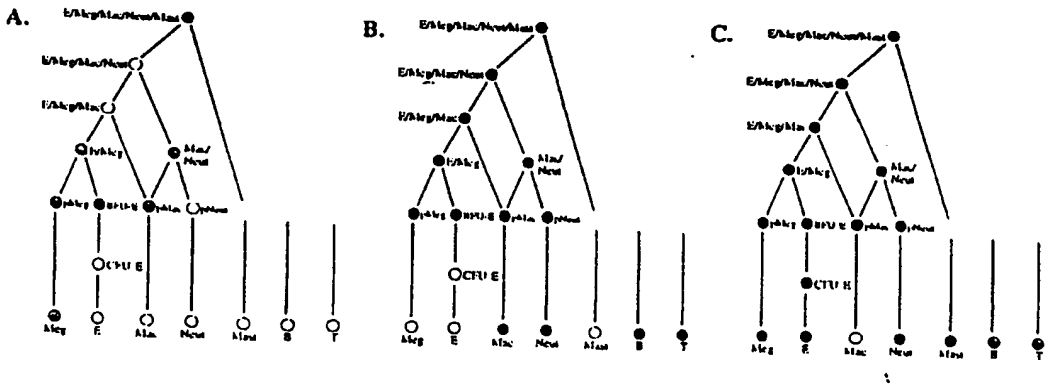
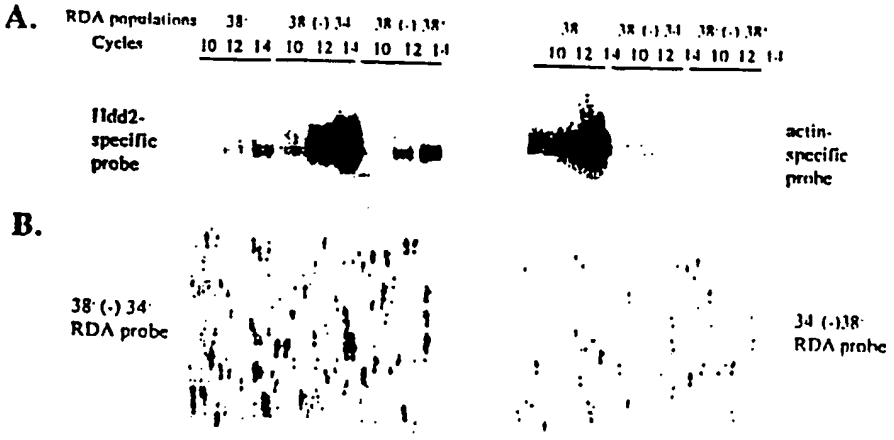


FIGURE 3



Duplicate arrays (3,000 clones) of full-length 38⁻ (-) 34⁻ library

FIGURE 4

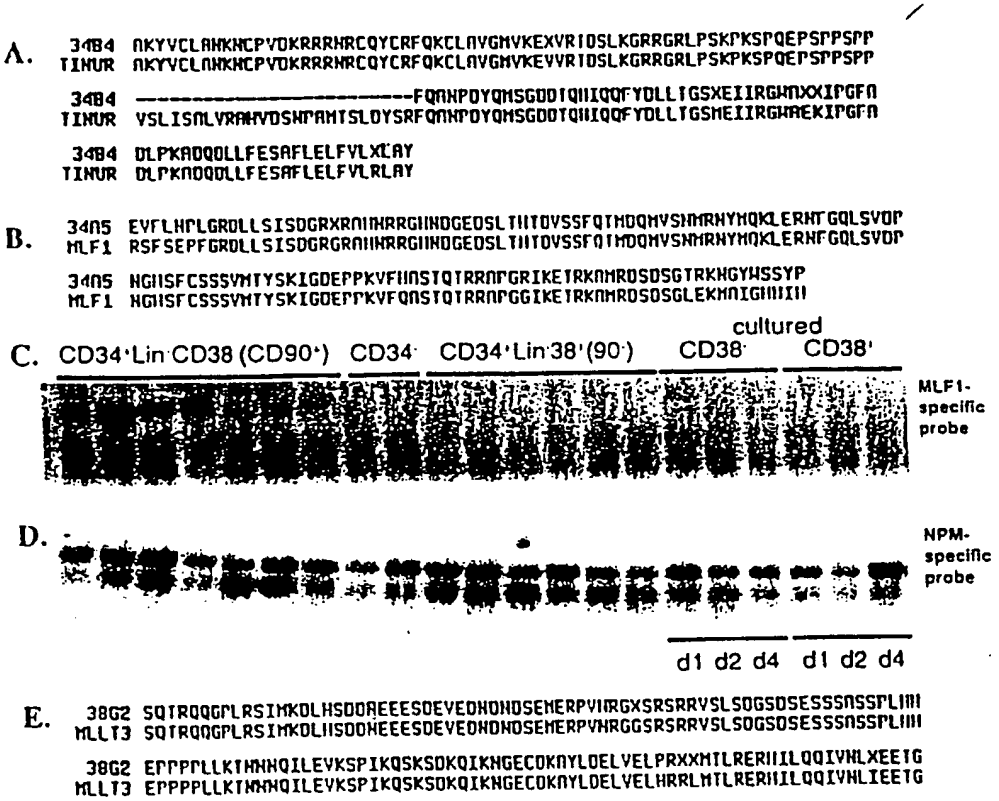

A.
34B4 NKYVCLNNKNCPVDKRRRNRCQYCRFQKCLNVGMVKEXVRTDSLKGRRGRLPSKPKSPQEPSPPSPP
TINUR NKYVCLNNKNCPVDKRRRNRCQYCRFQKCLNVGMVKEYVRTDSLKGRRGRLPSKPKSPQEPSPPSPP
34B4 ----------FQNNPDYQMSGDDTQHIQQFYDLLTGSXEIIRGWNXXIPGFN
TINUR VSLISNLVRNMVDSNTNMTSLDYSRFQNNPDYQMSGDDTQHIQQFYDLLTGSNEIIRGWREKIPGFN
34B4 DLPKNDQQLLFESNFLELFVLXCAY
TINUR DLPKNDQQLLFESNFLELFVLRLAY
B.
34N5 EVFLHPLGRDLLSISDGRXRNIHRRGIHNDGEDSLTHTDVSSFQTMDQMVSNMRNYMQKLERNTGQLSVDP
MLF1 RSFSEPFGRDLLSISDGRGRNIHRRGIHNDGEDSLTHTDVSSFQTMDQMVSNMRNYMQKLERNFGQLSVDP
34N5 NGHSFCSSSVMTYSKIGDEPPKVFHNSTQTRRNPGRIKETRKNMRDSDSGTRKNGYMSSYP
MLF1 NGHSFCSSSVMTYSKIGDEPPKVFQNSTQTRRNPGGIKETRKNMRDSDSGLEKMNIGHHIH
cultured
C. CD34+Lin-CD38-(CD90+) CD34- CD34+Lin-38+(90-) CD38- CD38+
MLF1-specific probe
D.
NPM-specific probe
d1 d2 d4 d1 d2 d4
E.
38G2 SQTRQQGPLRSIMKDLHSDDNEEESDEVEDNDNDSEMERPVHRGXSRSRRVSLSDGSDSESSSNSSPLHH
MLLT3 SQTRQQGPLRSIMKDLHSDDNEEESDEVEDNDNDSEMERPVHRGGSRSRRVSLSDGSDSESSSNSSPLHH
38G2 EPPPPLLKTMNNQILEVKSPIKQSKSDKQIKNGECDKNYLDELVELPRXXMTLRERHILQQIVNLXEETG
MLLT3 EPPPPLLKTMNNQILEVKSPIKQSKSDKQIKNGECDKNYLDELVELHRRLMTLRERHILQQIVNLIEETG

FIGURE 5

Figure 5.

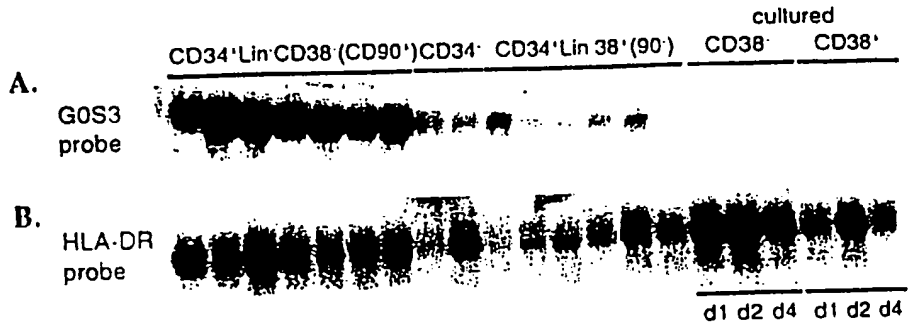


FIGURE 6

Figure 6.

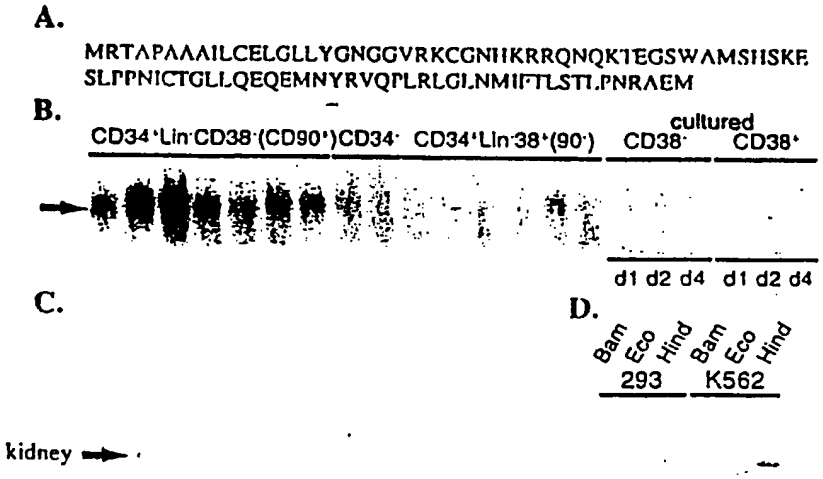


FIGURE 7

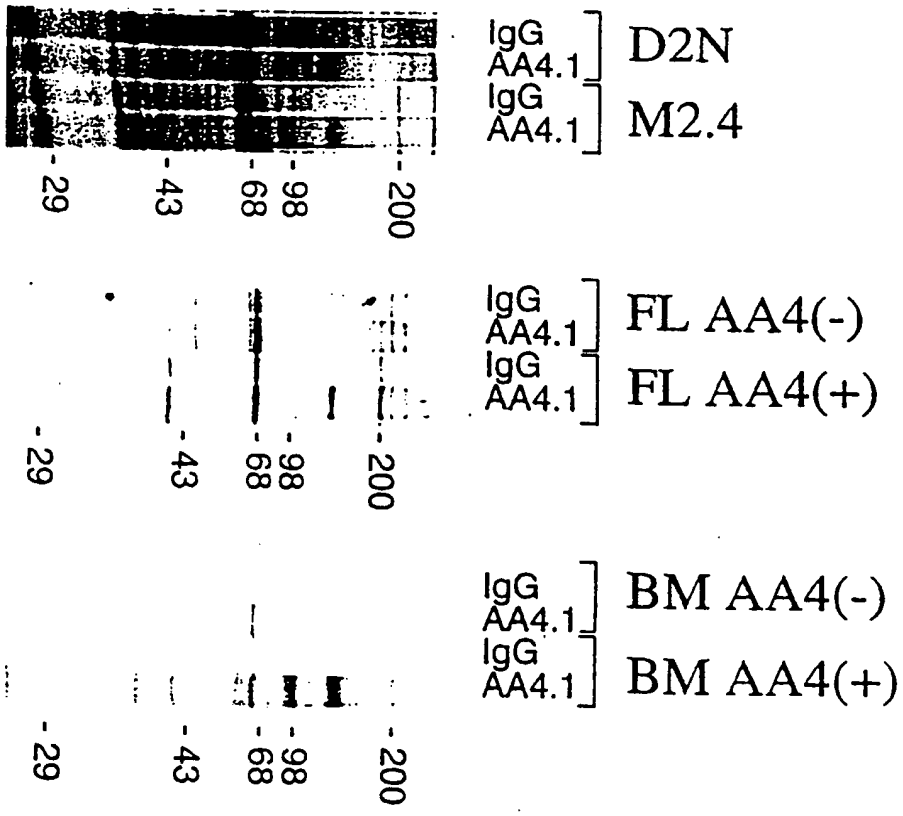

IgG
AA4.1
D2N
IgG
AA4.1
M2.4
29
43
68
98
200
IgG
AA4.1
FL AA4(-)
IgG
AA4.1
FL AA4(+)
29
43
68
98
200
IgG
AA4.1
BM AA4(-)
IgG
AA4.1
BM AA4(+)
29
43
68
98
200

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FIGURE 8

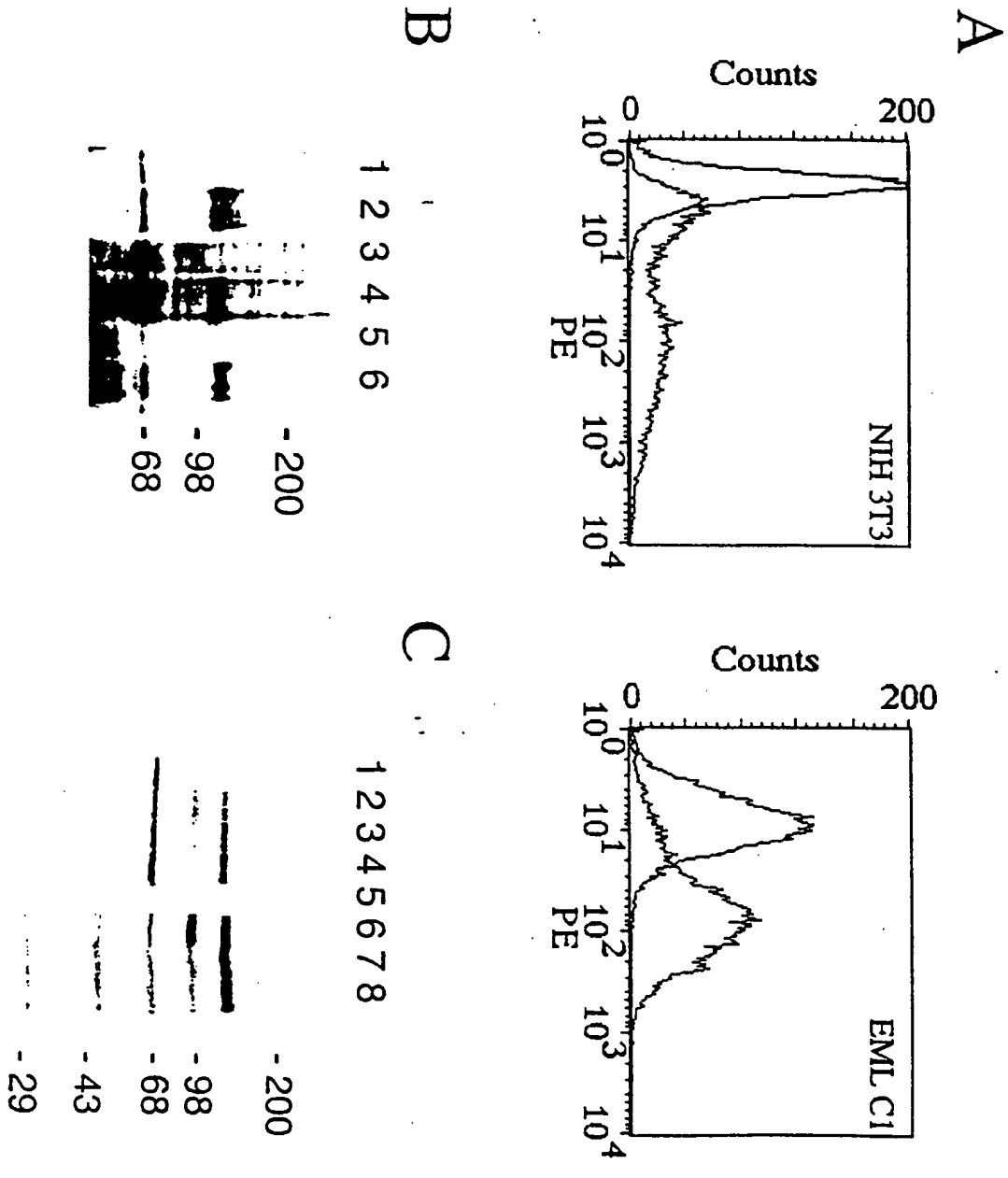
A
Counts
0
200
10^0
10^1
10^2
10^3
10^4
PE
NIH 3T3
B
1 2 3 4 5 6
- 200
- 98
- 68
C
1 2 3 4 5 6 7 8
- 200
- 98
- 68
- 43
- 29
Counts
0
200
10^0
10^1
10^2
10^3
10^4
PE
EML C1

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FIGURE 7

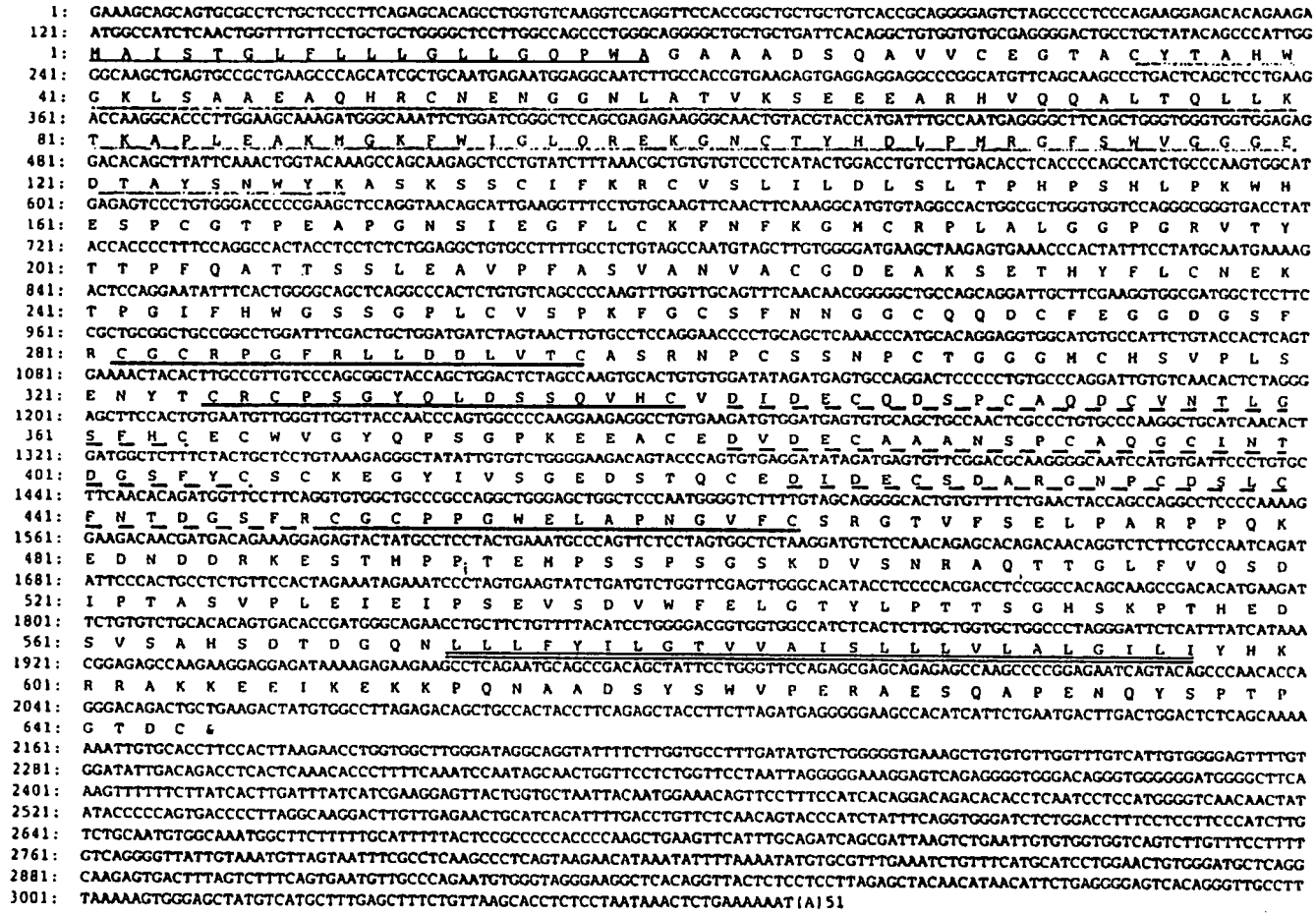
1: GAAAGCAGCAGTGCGCCTCTGCTCCCTTCAGAGCACAGCCTGGTGTCAAGGTCCAGGTTCCACCGGCTGCTGCTGTCACCGCAGGGGAGTCTAGCCCCTCCCAGAAGGAGACACAGAAGA
121: ATGGCCATCTCAACTGGTTTGTTCCTGCTGCTGGGGCTCCTTGGCCAGCCCTGGGCAGGGGCTGCTGCTGATTCACAGGCTGTGGTGTGCGAGGGGACTGCCTGCTATACAGCCCATTGG
1: M A I S T G L F L L L G L L G Q P W A G A A A D S Q A V V C E G T A C Y T A H W
241: GGCAAGCTGAGTGCCGCTGAAGCCCAGCATCGCTGCAATGAGAATGGAGGCAATCTTGCCACCGTGAAGAGTGAGGAGGAGGCCCGGCATGTTCAGCAAGCCCTGACTCAGCTCCTGAAG
41: G K L S A A E A Q H R C N E N G G N L A T V K S E E E A R H V Q Q A L T Q L L K
361: ACCAAGGCACCCTTGGAAGCAAAGATGGGCAAATTCTGGATCGGGCTCCAGCGAGAGAAGGGCAACTGTACGTACCATGATTTGCCAATGAGGGGCTTCAGCTGGGTGGGTGGTGGAGAG
81: T K A P L E A K M G K F W I G L Q R E K G N C T Y H D L P M R G F S W V G G G E
481: GACACAGCTTATTCAAACTGGTACAAAGCCAGCAAGAGCTCCTGTATCTTTAAACGCTGTGTGTCCCTCATACTGGACCTGTCCTTGACACCTCACCCCAGCCATCTGCCCAAGTGGCAT
121: D T A Y S N W Y K A S K S S C I F K R C V S L I L D L S L T P H P S H L P K W H
601: GAGAGTCCCTGTGGGACCCCCGAAGCTCCAGGTAACAGCATTGAAGGTTTCCTGTGCAAGTTCAACTTCAAAGGCATGTGTAGGCCACTGGCGCTGGGTGGTCCAGGGCGGGTGACCTAT
161: E S P C G T P E A P G N S I E G F L C K F N F K G M C R P L A L G G P G R V T Y
721: ACCACCCCTTTCCAGGCCACTACCTCCTCTCTGGAGGCTGTGCCTTTTGCCTCTGTAGCCAATGTAGCTTGTGGGGATGAAGCTAAGAGTGAAACCCACTATTTCCTATGCAATGAAAAG
201: T T P F Q A T T S S L E A V P F A S V A N V A C G D E A K S E T H Y F L C N E K
841: ACTCCAGGAATATTTCACTGGGGCAGCTCAGGCCCACTCTGTGTCAGCCCCAAGTTTGGTTGCAGTTTCAACAACGGGGGCTGCCAGCAGGATTGCTTCGAAGGTGGCGATGGCTCCTTC
241: T P G I F H W G S S G P L C V S P K F G C S F N N G G C Q Q D C F E G G D G S F
961: CGCTGCGGCTGCCGGCCTGGATTTCGACTGCTGGATGATCTAGTAACTTGTGCCTCCAGGAACCCCTGCAGCTCAAACCCATGCACAGGAGGTGGCATGTGCCATTCTGTACCACTCAGT
281: R C G C R P G F R L L D D L V T C A S R N P C S S N P C T G G G M C H S V P L S
1081: GAAAACTACACTTGCCGTTGTCCCAGCGGCTACCAGCTGGACTCTAGCCAAGTGCACTGTGTGGATATAGATGAGTGCCAGGACTCCCCCTGTGCCCAGGATTGTGTCAACACTCTAGGG
321: E N Y T C R C P S G Y Q L D S S Q V H C V D I D E C Q D S P C A Q D C V N T L G
1201: AGCTTCCACTGTGAATGTTGGGTTGGTTACCAACCCAGTGGCCCCAAGGAAGAGGCCTGTGAAGATGTGGATGAGTGTGCAGCTGCCAACTCGCCCTGTGCCCAAGGCTGCATCAACACT
361 S F H C E C W V G Y Q P S G P K E E A C E D V D E C A A A N S P C A Q G C I N T
1321: GATGGCTCTTTCTACTGCTCCTGTAAAGAGGGCTATATTGTGTCTGGGGAAGACAGTACCCAGTGTGAGGATATAGATGAGTGTTCGGACGCAAGGGGCAATCCATGTGATTCCCTGTGC
401: D G S F Y C S C K E G Y I V S G E D S T Q C E D I D E C S D A R G N P C D S L C
1441: TTCAACACAGATGGTTCCTTCAGGTGTGGCTGCCCGCCAGGCTGGGAGCTGGCTCCCAATGGGGTCTTTTGTAGCAGGGGCACTGTGTTTTCTGAACTACCAGCCAGGCCTCCCCAAAAG
441: F N T D G S F R C G C P P G W E L A P N G V F C S R G T V F S E L P A R P P Q K
1561: GAAGACAACGATGACAGAAAGGAGAGTACTATGCCTCCTACTGAAATGCCCAGTTCTCCTAGTGGCTCTAAGGATGTCTCCAACAGAGCACAGACAACAGGTCTCTTCGTCCAATCAGAT
481: E D N D D R K E S T H P P T E M P S S P S G S K D V S N R A Q T T G L F V Q S D
1681: ATTCCCACTGCCTCTGTTCCACTAGAAATAGAAATCCCTAGTGAAGTATCTGATGTCTGGTTCGAGTTGGGCACATACCTCCCCACGACCTCCGGCCACAGCAAGCCGACACATGAAGAT
521: I P T A S V P L E I E I P S E V S D V W F E L G T Y L P T T S G H S K P T H E D
1801: TCTGTGTCTGCACACAGTGACACCGATGGGCAGAACCTGCTTCTGTTTTACATCCTGGGGACGGTGGTGGCCATCTCACTCTTGCTGGTGCTGGCCCTAGGGATTCTCATTTATCATAAA
561: S V S A H S D T D G Q N L L L F Y I L G T V V A I S L L L V L A L G I L I Y H K
1921: CGGAGAGCCAAGAAGGAGGAGATAAAAGAGAAGAAGCCTCAGAATGCAGCCGACAGCTATTCCTGGGTTCCAGAGCGAGCAGAGAGCCAAGCCCCGGAGAATCAGTACAGCCCAACACCA
601: R R A K K E E I K E K K P Q N A A D S Y S W V P E R A E S Q A P E N Q Y S P T P
2041: GGGACAGACTGCTGAAGACTATGTGGCCTTAGAGACAGCTGCCACTACCTTCAGAGCTACCTTCTTAGATGAGGGGGAAGCCACATCATTCTGAATGACTTGACTGGACTCTCAGCAAAA
641: G T D C *
2161: AAATTGTGCACCTTCCACTTAAGAACCTGGTGGCTTGGGATAGGCAGGTATTTTCTTGGTGCCTTTGATATGTCTGGGGGTGAAAGCTGTGTGTTGGTTTGTCATTGTGGGGAGTTTTGT
2281: GGATATTGACAGACCTCACTCAAACACCCTTTTCAAATCCAATAGCAACTGGTTCCTCTGGTTCCTAATTAGGGGGAAAGGAGTCAGAGGGGTGGGACAGGGTGGGGGGATGGGGCTTCA
2401: AAGTTTTTTCTTATCACTTGATTTATCATCGAAGGAGTTACTGGTGCTAATTACAATGGAAACAGTTCCTTTCCATCACAGGACAGACACACCTCAATCCTCCATGGGGTCAACAACTAT
2521: ATACCCCCAGTGACCCCTTAGGCAAGGACTTGTTGAGAACTGCATCACATTTTGACCTGTTCTCAACAGTACCCATCTATTTCAGGTGGGATCTCTGGACCTTTCCTCCTTCCCATCTTG
2641: TCTGCAATGTGGCAAATGGCTTCTTTTTGCATTTTTACTCCGCCCCCACCCCAAGCTGAAGTTCATTTGCAGATCAGCGATTAAGTCTGAATTGTGTGGTGGTCAGTCTTGTTTCCTTTT
2761: GTCAGGGGTTATTGTAAATGTTAGTAATTTCGCCTCAAGCCCTCAGTAAGAACATAAATATTTTAAAATATGTGCGTTTGAAATCTGTTTCATGCATCCTGGAACTGTGGGATGCTCAGG
2881: CAAGAGTGACTTTAGTCTTTCAGTGAATGTTGCCCAGAATGTGGGTAGGGAAGGCTCACAGGTTACTCTCCTCCTTAGAGCTACAACATAACATTCTGAGGGAGTCACAGGGTTGCCTT
3001: TAAAAAGTGGGAGCTATGTCATGCTTTGAGCTTTCTGTTAAGCACCTCTCCTAATAAACTCTGAAAAAAT(A)51

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FIGURE 10

A

```
AA4     MAISTGLFLLLGLL-GQPWAGAAADSQAVVCEGTACYTAHWGKLSAAEAQHRCNENGGNL 59
        MA S GL LLL LL  QP AG  AD++AVVC GTACYTAH GKLSAAEAQ+ CN+NGGNL
C1qR    MATSMGLLLLLLLLLLTQPGAGTGADTEAVVCVGTACYTAHSGKLSAAEAQNHCNQNGGNL 60

AA4     ATVKSEEEARHVQQALTQLLKTKAPLEAKMGKFWIGLQREKGNCTYHDLPMRGFSWVGGG 119
        ATVKS+EEA+HVQ+ L QLL+ +A L A+M KFWIGLQREKG C     LP++GFSWVGGG
C1qR    ATVKSKEEAQHVQRVLAQLLRREAALTARMSKFWIGLQREKGKCLDPSLPLKGFSWVGGG 120

AA4     EDTAYSNWYKASKSSCIFKRCVSLILDLSLTPHPSHLPKWHESPCGTPEAPGNSIEGFLC 179
        EDT YSNW+K  ++SCI KRCVSL+LDLS     P+ LPKW E PCG+P +PG++IEGF+C
C1qR    EDTPYSNWHKELRNSCISKRCVSLLLDLSQPLLPNRLPKWSEGPCGSPGSPGSNIEGFVC 180

AA4     KFNFKGMCRPLALGGPGRVTYTTPFQATTSSLEAVPFASVANVACGDEAKSET--HYFLC 237
        KF+FKGMCRPLALGGPG+VTYTTPFQ T+SSLEAVPFAS ANVACG+  K ET  HYFLC
C1qR    KFSFKGMCRPLALGGPGQVTYTTPFQTTSSSLEAVPFASAANVACGEGDKDETQSHYFLC 240

AA4     NEKTPGIFHWGSSGPLCVSPKFGCSFNNGGCQQDCFEGGDGSFRCGCRPGFRLLDDLVTC 297
        EK P +F WGSSGPLCVSPK+GC+FNNGGC QDCFEGGDGSF CGCRPGFRLLDDLVTC
C1qR    KEKAPDVFDWGSSGPLCVSPKYGCNFNNGGCHQDCFEGGDGSFLCGCRPGFRLLDDLVTC 300

AA4     ASRNPCSSNPCTGGGMCHSVPLSENYTCRCPSGYQLDSSQVHCVDIDECQDSPCAQDCVN 357
        ASRNPCSS+PC GG  C   P  +NYTCRCP GYQLDSSQ+ CVD+DECQDSPCAQ+CVN
C1qR    ASRNPCSSSPCRGGATCVLGPHGKNYTCRCPQGYQLDSSQLDCVDVDECQDSPCAQECVN 360

AA4     TLGSFHCECWVGYQPSGPKEEACEDVDECAAANSPCAQGCINTDGSFYCSCKEGYIVSGE 417
        T G F CECWVGY+P GP E AC+DVDECA    SPCAQGC NTDGSF+CSC+EGY+++GE
C1qR    TPGGFRCECWVGYEPGGPGEGACQDVDECALGRSPCAQGCTNTDGSFHCSCEEGYVLAGE 420

AA4     DSTQCEDIDECSDARGNPCDSLCFNTDGSFRCGCPPGWELAPNGVFCSRGTVFSELPARP 477
        D TQC+D+DEC     G  CDSLCFNT GSF CGC PGW LAPNGV C+ G V     P+ P
C1qR    DGTQCQDVDECVGPGGPLCDSLCFNTQGSFHCGCLPGWVLAPNGVSCTMGPVSLGPPSGP 480

AA4     PQKEDNDDRKESTMPPTEMPSSPSGSKDVSNRAQTTGL-FVQSDIPTASVPLEIEIPSEV 536
        P +ED  +++ ST+P     S   G +        TT     + SD P  S PL++  PS
C1qR    PDEEDKGEKEGSTVPRAATASPTRGPEGTPKATPTTSRPSLSSDAPITSAPLKMLAPSGS 540

AA4     SDVWFELGTYLPT-TSGHSKPTHEDSVSA---HSDTDGQNLLLFYILGTVVAISLLLVLA 592
        S VW E  +  T  SG  +P   DS  A    +  TDGQ LLLFYILGTVVAI LLL LA
C1qR    SGVWREPSIHHATAASGPQEPAGGDSSVATQNNDGTDGQKLLLFYILGTVVAILLLLALA 600

AA4     LGILIYHKRRAKKEEIKEKKPQNAADSYSWVPERAESQAPENQYSPTPGTDC 644
        LG+L+Y KRRAK+EE KEKKPQNAADSYSWVPERAES+A ENQYSPTPGTDC
C1qR    LGLLVYRKRRAKREEKKEKKPQNAADSYSWVPERAESRAMENQYSPTPGTDC 652
```

B

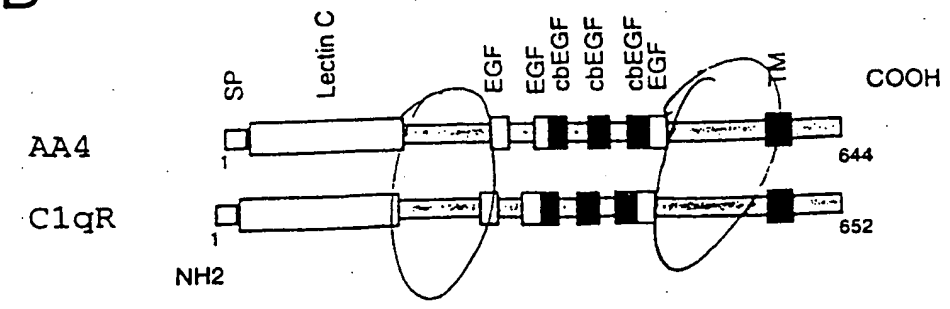

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FIGURE 11

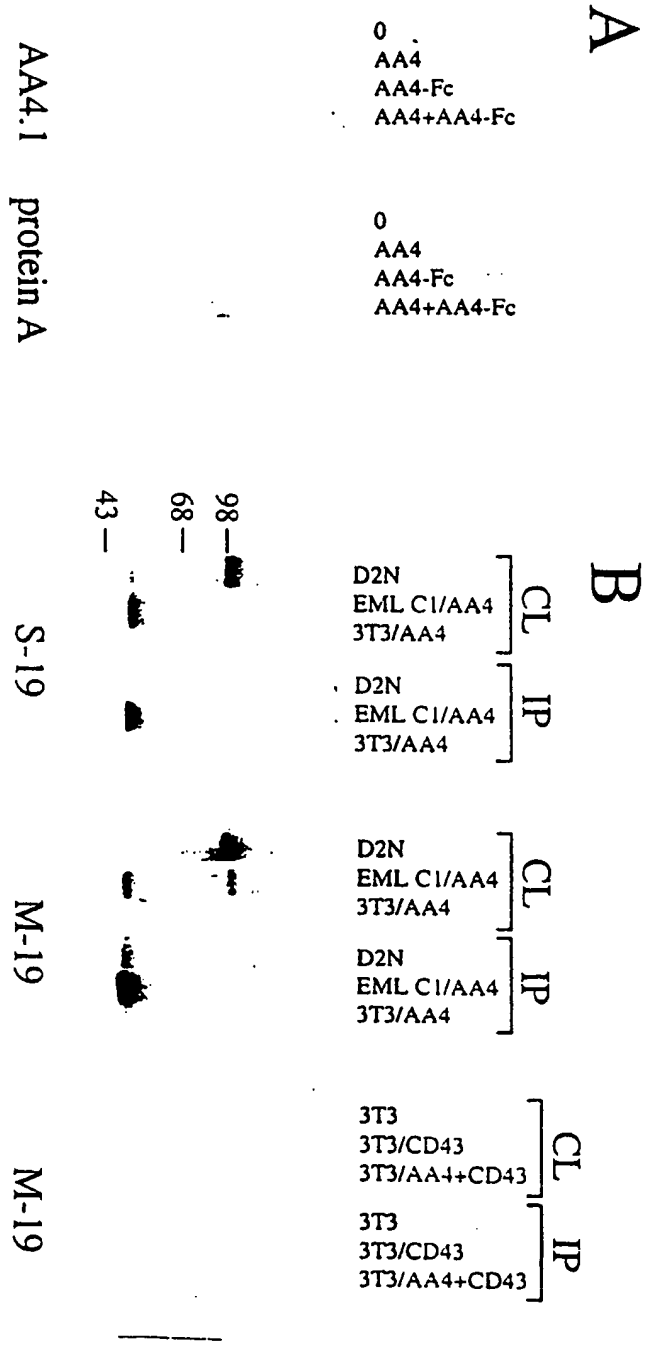

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FIGURE 12

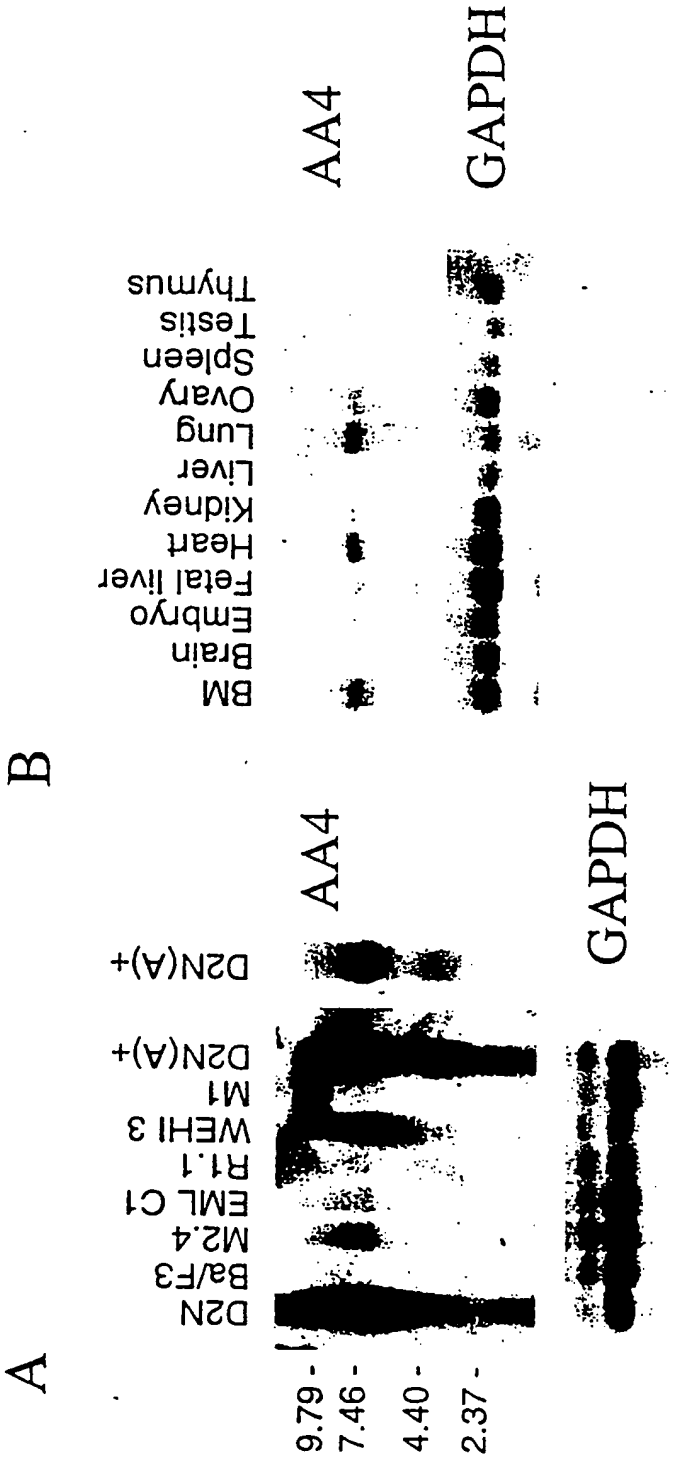

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FIGURE 13

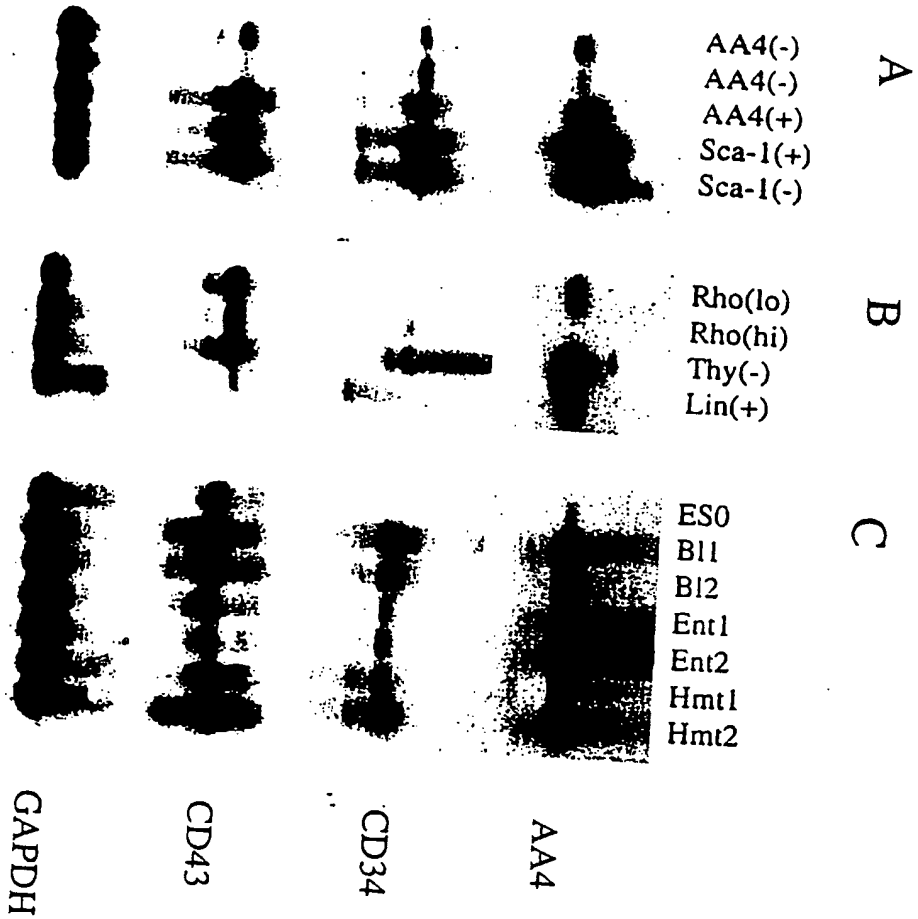

FIGURE 14

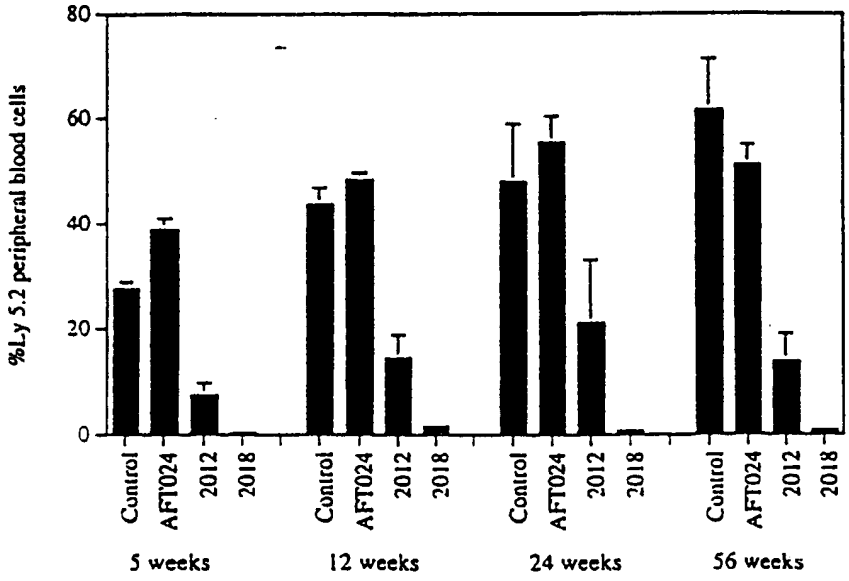
%Ly 5.2 peripheral blood cells
80
60
40
20
0
Control
AFT024
2012
2018
5 weeks
12 weeks
24 weeks
56 weeks

FIGURE 15

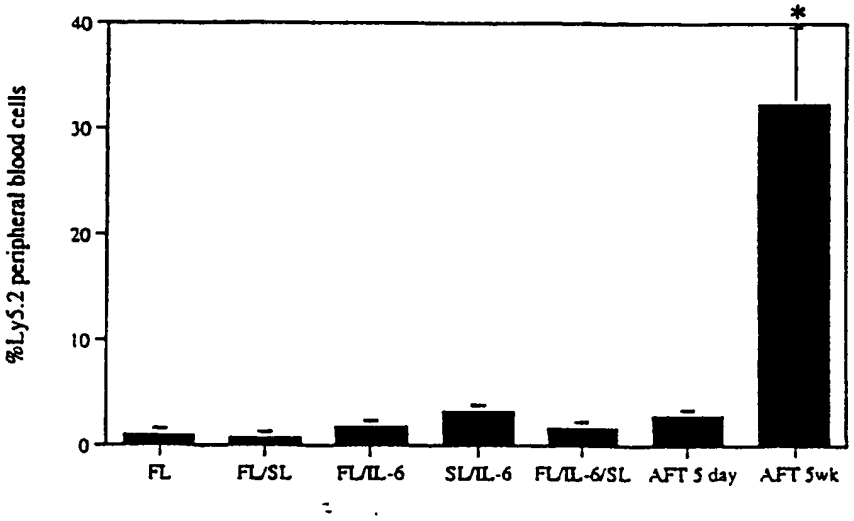
%Ly5.2 peripheral blood cells
40
30
20
10
0
*
FL
FL/SL
FL/IL-6
SL/IL-6
FL/IL-6/SL
AFT 5 day
AFT 5wk

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FIGURE 16

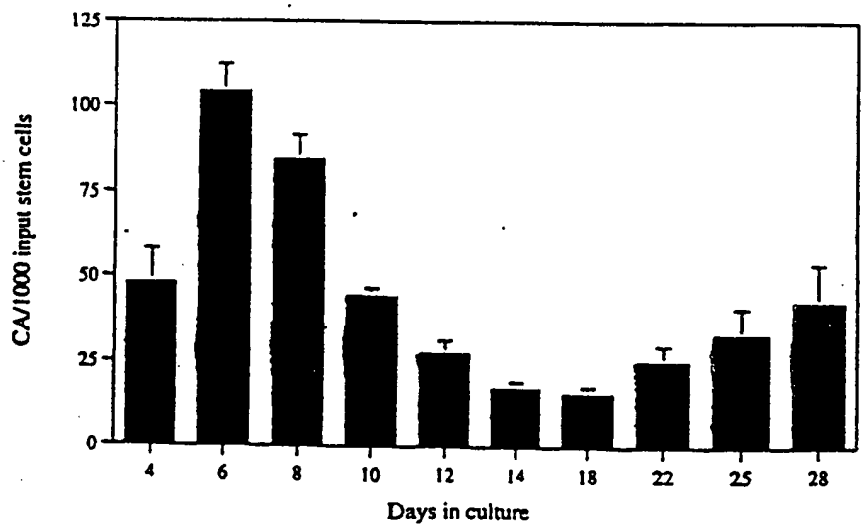

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FIGURE 17

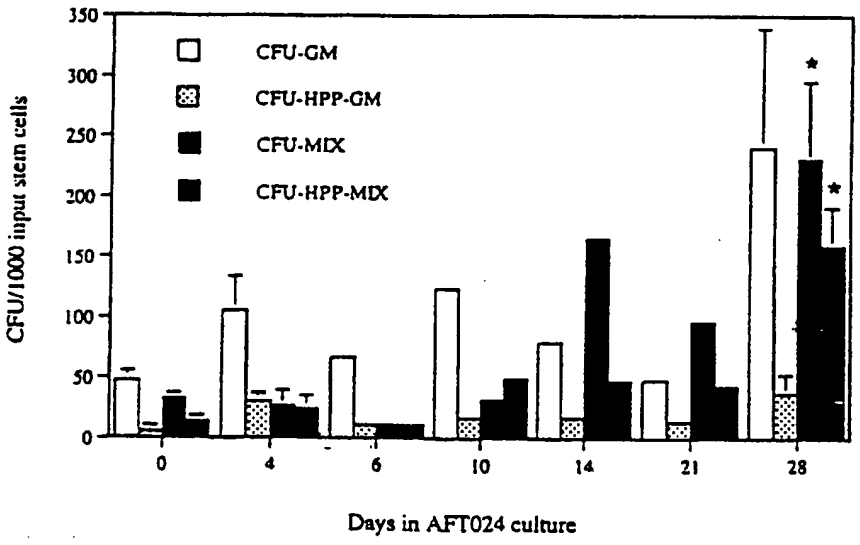

FIGURE 18

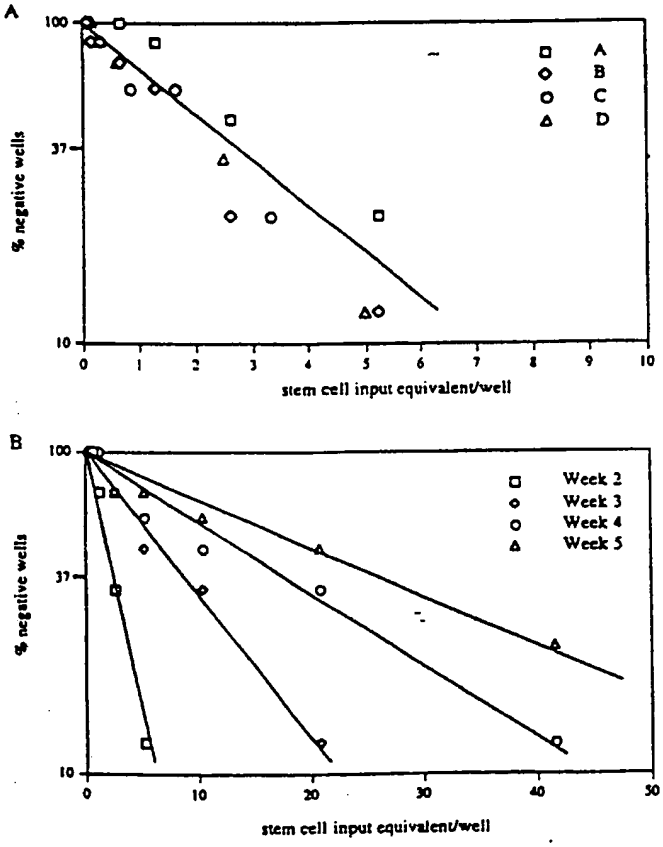
A
100
37
10
% negative wells
0
1
2
3
4
5
6
7
8
9
10
stem cell input equivalent/well
A
B
C
D
B
100
37
10
% negative wells
0
10
20
30
40
50
stem cell input equivalent/well
Week 2
Week 3
Week 4
Week 5

FIGURE 19

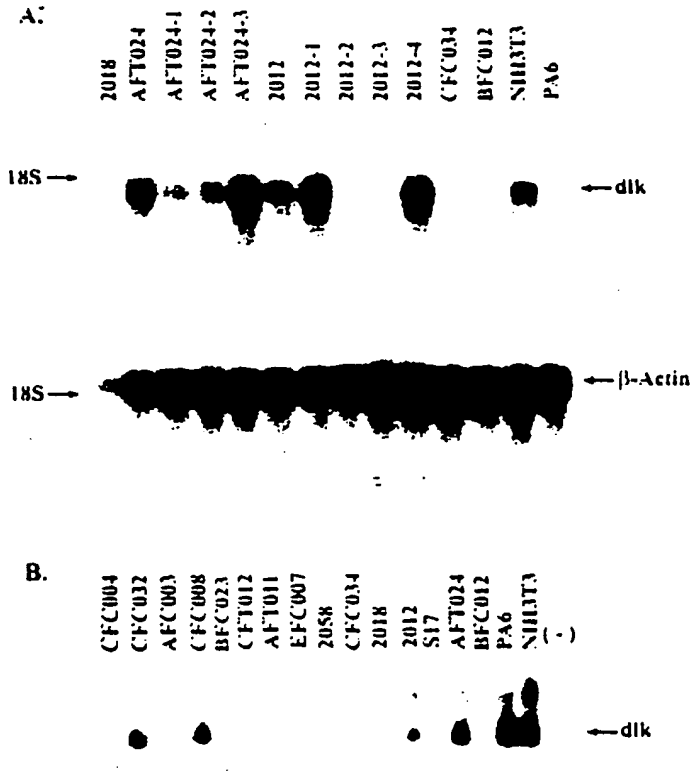
A.
2018
AFT024
AFT024-1
AFT024-2
AFT024-3
2012
2012-1
2012-2
2012-3
2012-4
CFC034
BFC012
NIH3T3
PA6
18S
dlk
18S
β-Actin
B.
CFC004
CFC032
AFC003
CFC008
BFC023
CFT012
AFT011
EFC007
2058
CFC034
2018
2012
S17
AFT024
BFC012
PA6
NIH3T3
(-)
dlk

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FIGURE 20

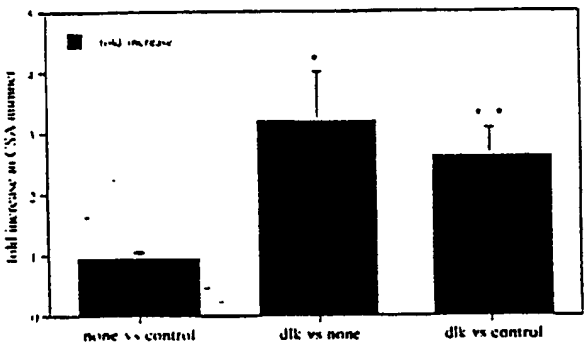

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FIGURE 21

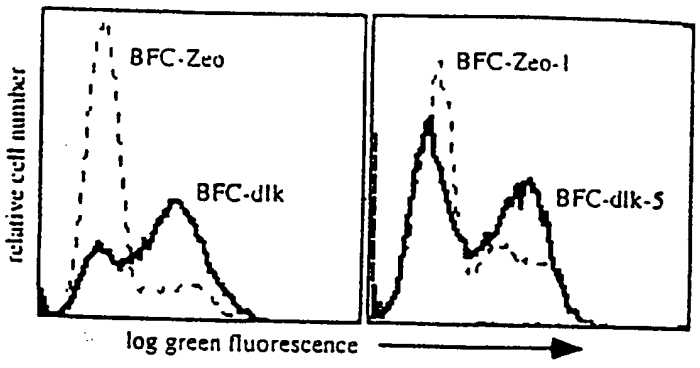

FIGURE 22

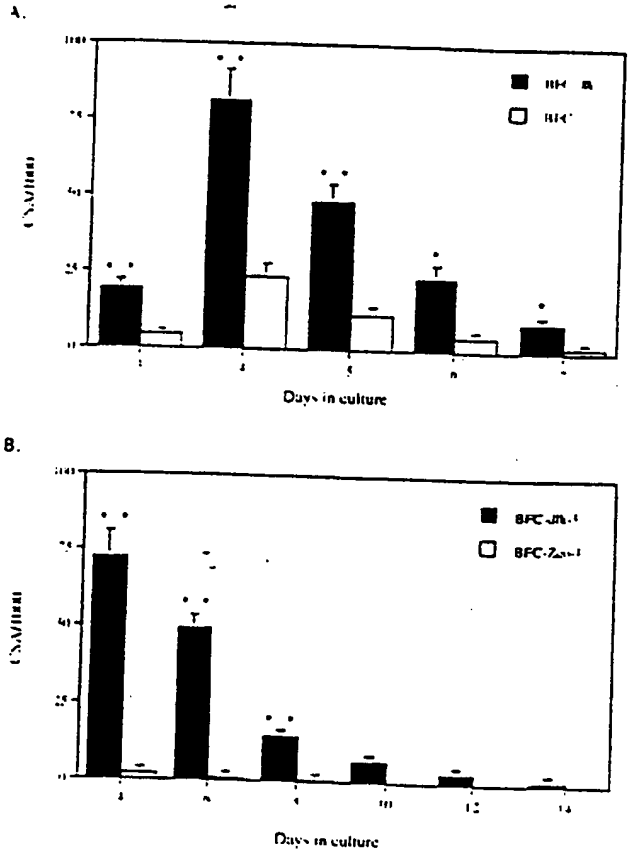

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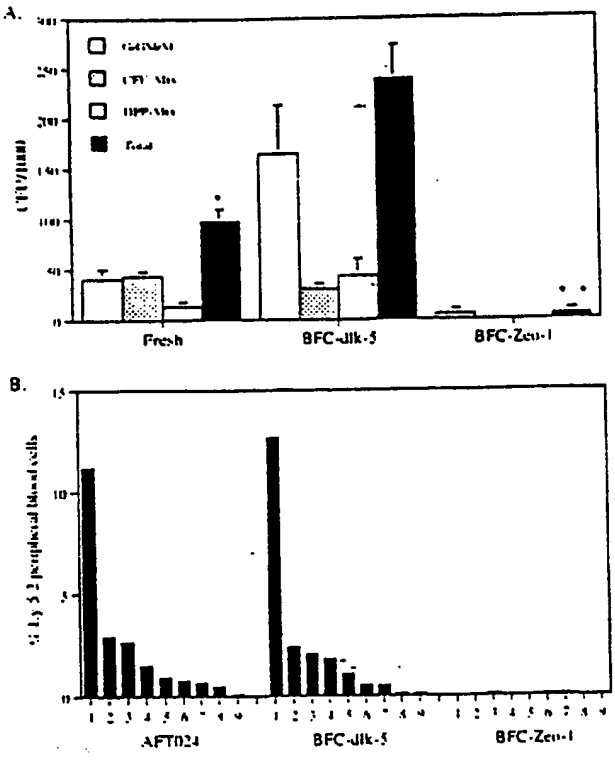
FIGURE 23



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LL4-39 (GL34)

Session Name: crick.princeton.edu 4


```
  1 CCCACGCGTC CGGAGAGCGG CAGCCATCTC CATGCCAAGG TTGAACAAAA
 51 TGCCAGCAAC GTTCTTTTCT GCTAGCCCAA AGGATTCGAA AGAACACAGC
101 CATTCTCTTC TAGACAACAA AAAGCAGAAA AAAAGGCCAA AGACTTTTGG
151 AATGGACGTC AAAACATACC TGAGATCGAT GATCCCACAT CTGGAATCTG
201 GGATGAAATC GGCCAAGTCC AAAGACATAC TTTCTGCTGA AGAAGTTATG
251 CAGTGGTCTC AGTCTCTGGA AAAATCCTTG CCAACCAGAC AGGTCAAAAT
301 GTCTTTGAA GATTCTAAAG TCTGATTCAG TGAGGAAAAT ATTGAATTCT
351 GGTTGGCTTC TGAGGACTAT KAAAAACAN AAACTGATCT TTTGCATANC
```

SEQ ID. No.: 1

FIGURE 24

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23.41 (D154)

Session Name: cri.ik.princeton.edu 4 Page 1

1 CCGCTGCTAG AAGACGACAG AAGGGGACTC ACTCGTTTTG AGAAGACCAT

51 GAAAGCGGCA GCCNTCTCCA TGCCAAGGTT GAACAAAATG CCAGGAATGT

101 TCTTTTTCTGC TANCCCAAAG GATTCGAAAN AACACAGCCA TTCTCTTC

SEQ ID. No.: 2

FIGURE 24

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BI-66 (related to Gag3B)

Session Name: crick.princeton.edu 4 Page 1

```
   1 TACGGCTCCC AGAAGACNAC AGAAGGGGAA NCAGCTGCGG TGGCCGCGGG
  51 AGTCTGACAA TGCAAAGTGC CATGTTCCTG GCTGTCCNGC ACGACTGCGT
 101 ACCCATGGAC AAGAGTGCAN GCAACGGCCC CAAGGTCNAN GAGAAGCGGG
 151 AGAAAATGAA TCGGACACTC TTNAANCATT GGAANACCCG TTTGAGCTAC
 201 TTCTNGCAGA GNNCCTCTGC TCCCGGGAAG CCCNGAACTG GCAATAAAAC
 251 NAACAGCATA CTTTTATGNA NCCTTCTCCT NANGAAGCGC AACTCTNGGC
 301 ATAAAGTTTC NATNAACTGC TGGCCNGTAT ATATGGGCTG GCTGCATTCA
 351 NGGCGTTTTC TAAAGTCCCA GTTCTATGAA GAAAACATTG AAATTCTGGT
```

SEQ ID. No.: 3

FIGURE 24

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LL2-12 (brainiac)

Session Name: crick.princeton.edu 1 Page 1

```
  1  GCCTGTTGGTG GCTCCGGAGC CCCTCCCTTC CTGCTCATCC TGGTGTGTAC
 51  GCCCCCGGAG CACCTGAACC AGAGAAACGC CATTCGGGCA TCTTGGGGTG
101  CCATCCCACGA AGCCCGGGGT TTCAGAGTGC AGACGCTCTT CCTCCTGGGA
151  AAACCTTGAA GACAGCAGCT TGCTGACCTG TCCTCAGAGT CAGCAGCACA
201  CAGGGATATC TTGCAGGCCT CCTTCCAGGA TTCCTACCGC AACCTCACCC
251  TCAAGAGCCT CAGTGGACTG AACTGGGTGA ACAAATACTG TCCTATGGCC
301  CGCTACATCC TCAAGACGGA TGATGACGTG TATGTCAACG TCCCAGAACT
351  GGTGTTCAGAG CTGATACAAA GAAGCGGCCC TTCGGACAAT GGCAGAAGCG
```

SEQ ID. No.: 4

FIGURE 24

LLS-96(lin).obl
Session Name: crick.princeton.edu 4


```
   1 CCCAAGCGTC CGATCTCCTC CAGGGCCACC AAGCACCTCT GAAGAGCCAT
  51 GTTCCAAGCC: GCCCGAGCCG CCCAGCCCAC CCCCTCTCAT GAAGCCAAAG
 101 GCAGCAGTGG: CAGCAGCACG GTACAGCGGT CTAAGTCCTT TACCTTGCGG
 151 CCTCAGGTGA AGGAGACCTG TGCAGCCTGC CAGAAGACTG TGTACCCGAT
 201 CGAGCCGCTG: GTGGCAGACA AGCTCATTTT CCACAACTCT TGTTTCTGTT
 251 GCAAACACTG: CCACACCAAA CTCAGCCTGG GCAGTTATGC TGCAATGCAC
 301 GGTGAATTTT: ACTGCAGACC TCACTTTCAG CAGCTGTTTA AGANTAAAGG
 351 CAACTACGAT GAAGGTTTGG TCGTAAACAG CACAAGGACT CTGGGCCAC
```

SEQ ID. No.: 5

FIGURE 24

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U.S.-96(Lin)SF

Session Name: crick.princeton.edu 1 Page 1

```
  1 ATTGGTNCCC CTGCAGGNAC CGGTCCGGAA TTCCCGGGTC GACCACGCGT
 51 CCGCCCNCCC GTCCGATCTC CTCCAGGGGG CACCAAGCAC CTCTGAAGAA
101 CATGTTCCAA GCTGCCGGAN CCGCCCANGC CACCCCCTCT CNTGAAGCCC
151 AANGCANCAN TGGCAGCAGC ACCGTNCAGC GGTCTAAGTC CTTTAACTTG
201 CGGCCTCAGT TGAAAGAANC CTGTGCAGCC TGCCNGAAAA CTGTGTNCCC
251 NATGGANCGT CTGGTGGCAG ACAANCTCNT TTTCCACAAC TCTTGTTTCT
301 GTTGCAAACA CTGCCACACC AAACTCANCC TGGGCAGTTA TGCTGCNATG
351 CACCGTTAAT TTTACTGCAG ACCTCACTTT CAGCAGCTGT TTNAGAATNA
401 AGGCNGCCAC NATGAAAGGT CTGGTCGTTA ACAGCACAAN GANCTCTGGG
451 CCCNCNTGGA AGTNGACTCA GCCCCNANAN AGGCTGANAA CCCTTTAACA
501 CCCATTNCCT CCCNNCACAT TGNCTCCCNC TNGGCAGTTG GAAAAGAAAA
551 TAANCCNNGG GCNCCCGGTT GGGANAAGAA AAAGGTCCCC CCCNCCGGTT
601 TTCCGGGNTA AGGGTCTCCC CCNNAATCCC TTCTTTTNCC CCTGGGAAGT
651 TTGGGTTTTG GAACCCAAAT TGGAATTTCC CCCTNCCGTN TTCCTTTNTT
701 CCCCNCCNCT CCCCCCCNNG GTCCCTGGGA AGGCCCCAAN CCN
```

SEQ ID. No.: 6

FIGURE 24

115-96 (sub ed)

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```
  1 GGGCGANTCT TGAGTCGCAT GGCCCGTTNC GTAAGCTTGG ATCCTCTANA
 51 ACGGGCNCCC TTTTTTTTTT GTTTTGCTGT TTAAATGTTT TTAAAAAGAA
101 GGTTGNTCAC CACAGAACNG CACNACNGAT GGAGGAGCAT ANCTNCTGCC
151 GACCCTGTCA GCTGANGCTC TAGCAANGAC TTTAGCTTTG TGGTGCAAGG
201 GACCCCNNAC CCTGAAAGCT GCAGGCTGTC TTGCCTCTTC CCCANCCCTC
251 CCCCCTCAGC GAAGTGGCAT CTATGAAGCC CAGTANNTGG CACTCTCCCT
301 CNTCCANTCT CTGTGGCTCA TTACCANGCT AANGGGCNGA NGTTNGTNGG
351 GANGCAAAGG GGTNCACNAT GCCCANGTGG CANTNAAGGA CTTGGGACCT
401 GAAGAAGCCA TGCTGACTCC TTCCTGCANA AAAAGCACCT AANTTCGAAC
451 TGGGCTTGTG GGCCTCCCNC GGAACGNTGG GGTGAGAANA AGTGGACTAT
501 AGGACCGTAT GGTGAAAATT GCAATTCTGG TTCCCAACCC NACCTCCCAT
551 GGGNAAAANT TAGGAATCCT GGAACCAAAC CCTTATGCCT GAAAACCTGT
601 TTTNAAGGCA NCCTCATCCT CTTCCCANCC CCGNGCCCCC NGGGGTTTAA
651 TCTNCCTTTC CCCCTGCCCC NNCCGGGGAA GGNCNNTTTT TCCTNGGGAA
701 AGGNAAAAAG GGTTTTTTTN AAAGGGGGTT CCCCNNGGCC CCCCNNTGGT
751 TNCCCTNGAT TTCCCCCCCC CCTNNTTTGG GCCCCNGAAA CCN
```

SEQ ID. No.: 7

FIGURE 24

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3449 PRI (seq)

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```
   1  ggatcccgg: taggaatgaa attagaagct gtagatcttca tggagccacg
  51  gttaatntg: gtagccacag ttactcgaat tattcaccat ctcttgagga
 101  tacatttttg: tggttgggaa caagagtatg accagtgggt agactgtgag
 151  tcccctgac: tctatcctgt aggg
```

SEQ ID. No.: 8

FIGURE 24

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scmgt

Session Name: crick.princeton.edu 1


```
   1 GGGGCTCANG GAAGCCGATC TTCCGGGTGG AGGGGGAAGC GGCGTGACTG
  51 GAGTNGAAAA TTTTCCCAAC ANAACTTCTC GGAGGCAACA TATTGGAAGG
 101 GACTCGGGGA GGCCGGAGTC CAAATGGAAG TGGCTGAAAG AAACTTCTCG
 151 CCCTNCTGAT TCTGAGCCCC GGGTCGTGCC GCGCGCCCTC AATTAGCTCA
 201 TCGAACTTTC GTCTTGACAG AACATTCTTC ACAATCCAAA AGAAAAGCA
 251 NACTNGTTCG GGATGTTTGA CGGCTATGAT AGCTGCAGTG AGGACACAAG
 301 TAGCNGCTCC AGCTCTGAGG AGAGTGAAGA AGAAGTTGCT CCTTTACCTT
 351 CCAATCTCCC AATCATCAAG AATAATGGAC AAGTCTACAC ATACCCAGAT
 401 GGTAAATCTN GCATGGCTAC CTGTGAGATG TGTGGGATGG TCGGTGTGCG
 451 AAATGCTTTT TACTCTAAAA CGAAACGTTT CTGCAGTGTT TCCTGTTCNA
 501 GAAATTACTC GTCCAACTCT AAGAAGNGAG CATTCTGGCC NGACTTCNNG
 551 TTACGGGTTN GCGTCCCACN AAGAAAGCCA AAATCCTTCC NAAACNACCN
 601 TTAGTTCCTA AATTNNCTGC CTATCCCCNT NTCCANCTAC CTTGCNGAAT
 651 CCNGCCAGAC NAAANCNGGC NATTCTGCCA TCTCTGTGGA AAGGGTCCCT
 701 GGGGGTTAC
```

SEQ ID. No.: 9

FIGURE 24

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24-20(carnichael)

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```
  1 TAGTGCTGCG ANAAGACGAC AGNAGGGTNC GGGCTCCGAN AAGACGACAG
 51 AAAGGGNGCT TTTCTCCGCT GGCCCCGGCG CGCCCGGCAG CTCCTCCCCG
101 GCCATGTTCG T TCACTTTCGC GGCCTTCTGC TATATGCTGG CGCTGCTGCT
151 CACCGCTGCG CTCATCTTCT TCGCCATCTG GCACATCATA NCNTTTGATG
201 AGCTGAAGAC CGACTACAAG AAACCCTATA GACCAGTGCA ATACCCTGAA
251 CCTCTTGTCC TTCCAGAAGT ACTCATCCAC GCGTTCTTCT GTGTCATGTT
301 TCTCTGTGCG CAGAATTGGC TGACCCTGGG CCTCAATATG CCCCTTTTGG
351 CNTACC
```

SEQ ID. No.: 10

FIGURE 24

C3-54 (related to c.pts 19)

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```
   1  TACGGCTGCA AGAAGACGAC AGAAGGGGAG TGCTCGCCGG TCCCAGCGTT
  51  GCCATACCAT CGAGATGGCA TCTCGTAGCC GGAGACCCGA ACACAGCGGA
 101  CCGCCGCAGC TGTTTTATGA CCAGAATGAA GCCCGGAAAT ACGTTCGCAA
 151  CTCACGCATG ATTGACATCC AGACCAAAAT GACTGAGCGA GCGCTGGAGC
 201  TCCTCTCTTT ACCAGAATGT CAGCCTTCTT ACCTGTTAGA CATTGGCTGC
 251  GGTTCTTCGG TGAGTGGAAA TTATATCTCA GAAGAAGGAC ACTACTGGGT
 301  GGGCATTGAA ATCAGCCCTG CCATGTTGGA TGCCGCCTTG GACGAGATAC
 351  AGGAGGTGAA TGCTGCTAAG GGACTGGCCA AGGCTCCTTT CAAACCGGCC
```

SEQ ID. No.: 11

FIGURE 24

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B4-14 (desig.)

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```
  1 ACGGCTNCGA GAAGACGACA GAAGGGTACG GCTCCGAGAA GACGACAGAA
 51 GGGTACNCCT CCGANGAGAC GACAGAAGGG TACGGCTCCG AGAAGACGAC
101 AGAAGGGCGA GGAAACTGTA AAAGCTATGC TGGGGANGCA GCGCGGANCT
151 TGATTCNCCT TCACCTGCTC TGGCCACCCG CTGACCCGGG GTTTCCGGCC
201 GGANAGCNGT CANTATGAA CGGACGGGTG GATTATTTAG TCACGGAGGA
251 AGAGATCNAC CTGACGAGAG GACCTTCGGG GCTGGGCTTC AACATCGTCN
301 GTCCGANNGA TCAACAGTAT GTCTCCAACG ACAGTGGCAT CTACNTCAGC
351 CGTATCNAAN AAGATGGGGC TGCNGCCCAN GATGGGCGGC TCCAGGAAGG
```

SEQ ID. No.: 12

FIGURE 24

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A7


```
   1 AGCTCTAGTC CCCAGAGATG TCGCCACTAC TGCTGCTGCT GCTGTGCCTG
  51 CTGCTGCGGA ATTTGGAGCC TGAGGAGGCC AAACTGATCC GTGTCCCTCT
 101 TCAACGAATC CACCTTGGAC ACAGAATCTT AAACCCACTG AATGGATGGG
 151 AACAGCTGGC AGAGCTTTCT AGGACCTCCA CCTCTGGTGG CAACCCCTCC
 201 TTTGTGCCTC TCTCCAAGTT CATGAACACC CAGTATTTTG GAACTATTGG
 251 TTTGGGAACG CCTCCTCAGA ATTTCACCGT TGTCTTTGAC ACGGGTTCTT
 301 CCAACTTGTG GGTTCCGTCC ACGAGATGTC ATTTCTTCAG TTTGGCATGC
 351 TGGTTTCACC ATCGCTTTAA TCCCAAGGCC TCCAGCTCCT TCAGGCCCAA
 401 TGGGACCAAG TTTGCCATTC AGTATGGGAC CGGCGGCTG AGCGGAATCC
 451 TGAGCCAGGA CAATCTGACT ATCGGGGGCA TCCACGATGC TTTTGTGACA
 501 CTTGGAAGAG CTCTGTGGGA GCCCAGCCTG ATCTTTGCTT TAGCCCACTT
 551 TGATGGGATC CTGGGCCTCG GCTTCCCCAC TCTGGCTGTG GGCGGAGTTC
 601 AGCCTCCGCT GGATGCGATG GTGGAGCAAG GGCTGCTGGA GAAACCCGTC
 651 TTCTCCTTTT ACCTCAACAG GGATTCTGAA GGGTCTGATG GGGGAGAGCT
 701 GGTCCTGGGG GGCTCAGACC CCGCTCACTA CGTACCTCCC CTCACCTTCA
 751 TACCAGTCAC CATCCCTGCC CACTGGCAGG TCCACATGGA GAGTGTGAAG
 801 GTCGGCAGAG GGCTGAGCCT CTGTGCCCAG GGCTGCAGTG CCATCCTAGA
 851 CACAGGGACA TCCCTCATCA CAGGACCTAG TGAGGAGATC CGGGCCTTGA
 901 ATAAAGCCAT TGGGGATAT GCCTTCCTGA ATGGGCAGTA CTTCATTCAG
 951 TGTCCCAAGA CGCCAACGCT TCCCCCTGTC TCCTTCCACC TTGGTGGAGT
1001 CTGGTTTAAC GTCACAGGCC AGGACTATGT CATCAAGATT CTTCAGAGCG
1051 ATGTTGGCCT CTGCCTGTTG GGCTTCCAAG CCTTGGATAT CCCCAAGCCT
1101 GCGGGACCCC TCTGGATCCT TGGGGACGTC TTTTTGGGGC CCTATGTGGC
1151 TGTCTTTGAC CGTGGGGACA AAAACGTCGG CCCCCCCGTG GGACTGGCGC
1201 GTGCTCAGTC TCGTTCAACA CACCGGGCAG AAAGAAGGAC TACGCAGGCG
1251 CAGTTCTTTA AAAGACGCCC TGGTTAGGGT ACAAGCTCAC CGGGCCACAG
1301 CAGCTATGCT TCTTTCCAAT TAAACAAACT AAAAAAAAAA AAAAAAAAAA
```

SEQ ID. No.: 13

FIGURE 24

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Cyt-19 Page 1

```
   1 TCTTCGAAAG CCGGGCTGAG GGGAATCCTG GACAGGGGAA TCCTGGACGT
  51 GGAGATCGTG AGTCATGGCT GCTTCCCGAG ACGCTGATGA GATCCACAAG
 101 GACGTTCAGA ACTACTATGG GAATGTAGTG AAGACATCTG CAGACCTCCA
 151 GACTAATGCT TGTGTCAGGC CAGCCAAGCC GGTCCCCAGC TACATCCGGG
 201 AAAGTCTTCA GAATGTACAC GAAGACGTTA GTTCGAGGTA TTATGGCTGT
 251 GGTCTGACTG TTCCTGAGCG GCTGGAAAAC TGCCGAATTT TGGATCTGGG
 301 TAGTGGCAGT GGCAGGGATT GCTATGTGCT TAGCCAGCTG GTTGGTGAGA
 351 AGGGACATGT CACCGGAATA GACATGACTG AGGTCCAGGT CGAAGTGGCT
 401 AAAACCCATC TTGAACAGCA CATGGAAAAA TTTGGTTTCC AGGCACCCAA
 451 TGTGACTTTT CTCCACGGCC GCATCGAGAA GTTGGCAGAG GCTGGGATCC
 501 AGAGTGAGAA CTATGGTATT GTCATATCCA ACTGTGTTAT CAACCTTGTT
 551 CCTGATAAAC AACAAGTCCT CCAGGAGGTC TATCGAGTGC TGAAGCACGG
 601 CGGCGACTC TATTTCAGTG ACGTCTATGC CAGCCTTGAA GTGCCAGAAG
 651 ACATCAAGTC GCACAAAGTT CTATGGGGGG AATGCCTGGG AGGCGCTCTG
 701 TACTGGAAGA ATCTTGCCAT CATTGCCCAA AAGATTGGGT TCTGCCCTCC
 751 ACGTTTTGTC ACTGCCGATA TCATTACTGT TGAAAACAAG GAGCTCGAAG
 801 GGGTTCTTGG TGACTGTCGC TTTGTGTCTG CCACATTTCG CCTCTTCAAA
 851 CTCCCTAAGA CAGAGCCAGC CGAAAGATGC CGAGTTGTTT ACAATGGAGG
 901 AATGAAGGA CATGAAAAGG AACTAATTTT CGATGCAAAT TTCACATTCA
 951 AGGAAGCGA AGCTGTTGCA GTGGATGAGG AGACGGCAGC TGTCCTGAAG
1001 AACTCACGTT TTGCTCCGGA TTTTCTCTTC ACACCTGTTG ACGCCTCGCT
1051 GCCAGCTCCC CAGGGGCCGT TGTGAGTTAG AGACAAAGGT TCTAATCAGA
1101 GATTCATTCA AGCTTGCAGA GGACTCTGAC AAGATGAAGC CCAGACATGC
1151 ACCTGAAGCC ACGGGAGGCT GCTGTGGCAA GAGGAAAAAC TGCTAGATCT
1201 ACAGCCAGCG CGGAGCCCAC CGGGCTCAAG AGGGTGGCTA AAGGACAGTC
1251 ACAAAGGCTT CTTAGCCTGC TCTTCGCCAG TGCACAGATT ATGTGAAGGT
1301 GGCAAAGCCA CCACAAGCTA GACCACTGCT AAGAATAAGA GTGACTTTTA
1351 GAGAATGTTA ATTGAAGGTT CACAGCAAAT CGCCTGCTTT TCTATTTCTC
1401 TATTTCAGAG TTCTGGTGCC ACCTAGTGGT CAGAAGTAGA ACTTGGAAGC
1451 CCAAGGTTTA CTCAAAGGGC CAAAGCATC ATCAACGTTG TGAGAATTAT
1501 CTTCCTTCTG GCCTACCACA GGACACCTCT GGGTTCTTCT CTGTGGTTAC
1551 CAGAAAGCAC AGTACTTACT AAATTTATGC TAACCATGAC AAAAGATTGT
```

SEQ ID. No.: 14

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Cont-Page 2

1601 CAAC.CAAAT TTGCTAGGAG TATTCTTTAG GTTCCTGTCT GCAATTTTTT

1651 TCTC.TGTAAC TGAATGAAAA AGAAAACAAA TAAAAAATAA ATTTGACTTC

1701 GAAA.AAAAA. AA

SEQ ID. No: 14 cont'd

FIGURE 24

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```
   1 CGGAGCGCTG GGGGAGTCTG TGGAGCCCCC GGTCAAAGAC GGCATCCTCT
  51 ACCAGCAGCA CGTAAAGTTT GGCAAGAAAT GCTGGCGCAA AGTGTGGGCT
 101 CTGCTGTATG CGGGAGCCCC ATCAGGGGTA GCTCGGCTAG AAAGCTGGGA
 151 CGTGCGTGAT GGTGGCCTGG GACCAGCAGG CGACAGGTCC ACAGGGCCCA
 201 GCCGTCGAGC GGAACGCCCG GTCATACGCT TGGCTGACTG TGTATCTGTC
 251 CTGCCTCCGC ATGGCGAGAG CTGTCCCAGG GACACTGGTG CCTTCCTGAT
 301 TACCACCACC GAGCGAAGCC ACCTGTTGGC TGCACAGCAC CGCCAGTCCT
 351 GGGTGGAGCC CATCTGTCAG CTGGCCTTCC CGGGTACCGG AGAATGTTCG
 401 TCAGGATCAG GACAGGCTGA GAATCCAAAA AGGGGCTTTG TTCCCATGGA
 451 AGAAAACTCT ATCTACTCCT CCTGGCAGGA AGTGACCGAA TTTCCGGTGA
 501 TGGTGCAGAA GACAGAAGCC ACCTCCCGCT GCCAGCTGAA AGGACCCTAC
 551 CTCCTGCTGC TGGGCCAAGA TGACATCCAA CTGAGGGAGA CATCCAAGCC
 601 CCAGGCCTGT TTTAGCTGGC CCTACCGTTT CCTGCGCAAG TACCGCTCTG
 651 ACAAGGGTGT GTTCTCGTTT GAGGCTGGCC GCCGCTGTGA CTCAGGTGAG
 701 GGCCTTTTTG CCTTCAGTAG CCCGCGTGCC CCAGACATAT GTGGGGTTGT
 751 GGCTGCCGGC ATTGCCCGCC AGCGGGAGCG TCTTCCAGAG CTGGCCATGT
 801 CCCCAGCCTG CCCCCTGCCT CGGGCCCTCT CCCTGCCCTC CCTAGAGCCC
 851 CCTCGAAGAC TTCGGGAGGT GGCCCCAGGA TTTGAGCTGC CCACTCCCAG
 901 AAAGCTGCCT CTAACTGATC CCGGGCCTCA AAGCCTAGCA TTGCTGCTCA
 951 GCCCCAACAC AAGAACGACC GGCATCCCGT CTCTATGCGT CCGTGTGCAA
1001 GCAGAGCAGC AAGCACACAG GCACGGCGCA GCATTTCTAT GAGAACGTGT
1051 GCATGCTGGA GGCCAGCCTT GGGCTGACCA ATGGGGGTCC TGAAGCCCAA
1101 GAGGGCCCCC TTGGTGGCCG CAGCCCCCTT GGGCAGCCTT ATCTACCATA
1151 ACACTGAGGA TCTGAGTTGG CCGGGCTCGG CCCAGGACAG CAATCTGGAA
1201 GCCCAGTACC GGAGGCTGCT GAACTGGAG CTGGATGAGG CCGGAAGCGC
1251 CGGCCGTTCT GGAGCGCAGG CAGGCATCAA GGCCAAGCTG GTGACCCTGC
1301 TGAACCCTGA ACTGAAGAAG GCCCCCCGCC CCTGTGACCG GCCCTGAAGG
1351 CCTGAGTGGC CAGCCACTGC AGGACAGAGG TGATCACCCA AGACCAGGAA
1401 CAACTTTCGA CATAACCCGT CTACTCTGAC CTGCAGGGAC AAGCCAGGTG
1451 GCCCGGGGAG GAGCCACACT CTGCCCTACC TCCTCCCTCA GACTGTACAG
1501 ATTGAACAAT AATAAACCTT CCTATCAAC TTCAAAAAAA AAAAAAA
```

SEQ ID. No. 15

FIGURE 24

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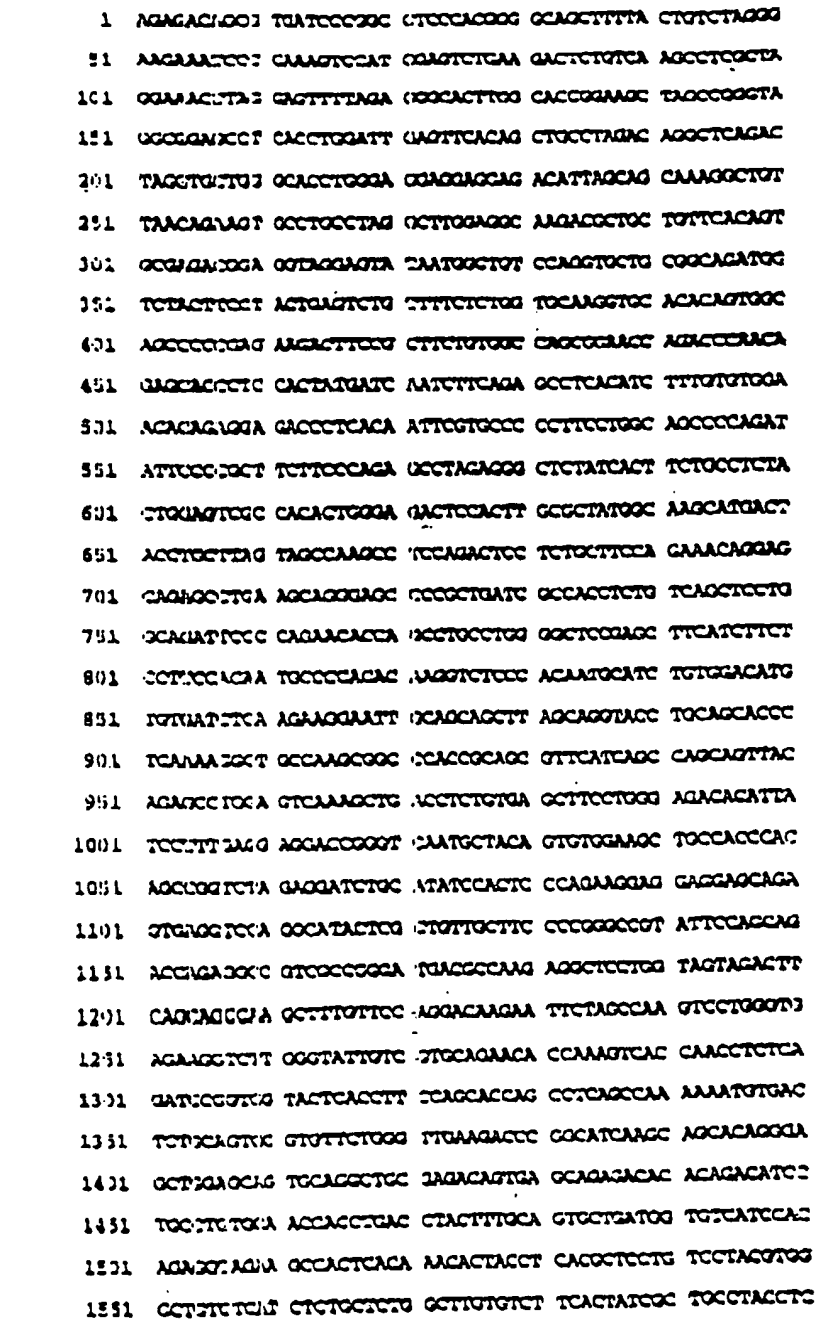

SEQ ID. No.: 16

FIGURE 24

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1601 TGCTCCAAGA GGAAGTCACG TAACTACACC ATCAAAGTCC ACATGAACCT
1651 GCTGTCCGCT GTCTTCCTGC TGGACGTGAG CTTCCTGCTC AGCGAGCCTG
1701 TGGCACTTAC GGGCTCCCAA GCAGCCTGTC GCACCAGTGC CATGTTCCTG
1751 CACTTCTCCC TGCTTGCCTG CCTCTCCTGG ATGGGCCTCG AGGCTACAA
1801 TCTCTACCGA CTGGTGGTGG AGGTCTTCGG TACCTATGTG CCCGGCTATC
1851 TGCTGAAGCC GAGCATCGTG GGCTGGGGTT TTCCTGTCTT CCTGGTCACT
1901 CTGGTGGCGT TCGTGGATGT GAATAACTAC GGCCCCATTA TCCTAGCTGT
1951 GGGCCGGACT CCGGAACGTG TCACCTACCC CTCTATGTGC TGGATCCGGG
2001 ACTCCCTGGT GAGCTATGTC AGCAACCTGG GCCTCTTCAG TCTGGTGTTC
2051 CTGTTCGTAC TGGCTATGCT GGCCACCATG GTGGTGCAGA TCCTCGGCT
2101 TCGCCCTCAA AGCCAGAACT GAGCCCACGT GCTGACCCTG CTGGGCTCA
2151 GCCTGGTCCT TGGCCTCCCC TGGGCTTGG TCTTCTTTTC CTTTGCTTCC
2201 GGCACCTTCA AGCTTGTCAT CCTCTACCTC TTCAGCATCA TAACTTCCTA
2251 CCAAGGCTTT CTCATCTTCC TGTGGTACTG GTCCATGCGG TTCCAGGCCC
2301 AAGCGGGCCC CTCCCCTCTG AAGAACAACT CAGACAGCGC CAAACTCCCC
2351 ATCAGCTCCA GCAGCACCTC CTCCAGCCGC ATCTAAGCCA CCCCCACACC
2401 TCCCCTTCGA GAGGACACAT GCATGGCGTC CGCTCACGAT GTCTGTGGCC
2451 CAGTGCTGTA CCCACCCAGC CTTTGTTGGT TAGTGGCATA CTAGAGAAGG
2501 CCCTGGTCCT TGAAGGCGTA GGGCTGTTGC TCTGGTAGGT AGATACCTAG
2551 GTTCCCTTGG GGACGACTCT GGTCCTCAAA GGGCTCAGAA GCACACTGCC
2601 ATTCTGTTTG TGGGGCCGTT TCAGTCTGGA GCTAAGGCCT TGTCTTTCTG
2651 GCCACCTCTG GGTCCAGCTG TTGCTGCTGG GTGTTGAGAC CTGCAGACCC
2701 AAGCTGCGGT TAGATCTCGA AGGAGGCTGA CACATCCGGC CTGAGACACA
2751 GCTAACTGTC TTGACTTGCT GCTCTGTCTC TGTGGTCACC ATGCAGATCC
2801 CGAGGGTGCC ACTGGGGGTA AATGTTCTGG GAGAAGGTTT GGAGGCAGAG
2851 CAGCTTTAGA GCTGAGCATC TCCCCCAGCC TTTGTGCAAA CCCTCCTCTT
2901 CATTCCCCAT CCCCAACCCC TCCTCTGTGT TCCCCTAACC CTCCACCTGA
2951 AGCCTTGGGT CCTAGACCAA TGCTGTGATT TGGGGTGGTA GTTCCCAGCA
3001 GTTTCCTGAT GCCAGCTATC AACTTCTGTC TGTTGTGTGG GCTTTGGCGT
3051 CTGACTCAAG GCAGGTTTCT GTCTGAGCCC TCTCTCCAAG CTGCCTCACC
3101 TTTGCTCCCA CCTCAGAGGG ACCTCCATCT CTCCTGAAGC CTCCTCCCTC

SEQ ID. No: 16 (cont'd)

FIGURE 24

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3151 TGGCTAGTAC: TGGGATACAG CCACCCTTTC AACCCAGCAC TCTGAAGACC
3201 AAGACAGCCC: CCTCTGGTGA CACTGGCCAA GCTTGATCTT TTTCCTAAGA
3251 AGTGGTCTTT: AGATCCCCGC AGGTCGCTCA GAAGACACTG GGCTGCCTAG
3301 TGTGAATTCC: GTCCTACTAA CGTACAGTGA GCAGCTCCTC ACCCCCACCC
3351 CCGCAAAAGC: TCTCACCAAG TCCTGGAGTG TCAGGCAGGG GGCTGGAAAT
3401 CCAGGAGGAC: TTCCTGCAAA AGGCAGCATT TCATCTTGAC CTCAGCCTTC
3451 AGGTTCCCGG: GAATGTTCTT TTTAAATACC AGTTCATTTG TCTTTTGATA
3501 TTAAAGCTCT: TTATAGAGAG TCTGGAAACT GTAGGCGATT GTCGAGAAGA
3551 GAAATAAAAA: TGAGCTGTTA TCTAATGCCA TGGCAAAGCA GCACAAAAA

SEQ ID. No: 16 (cont'd)

FIGURE 24

AFTP2 BMP/ TGFR/ PDA

Sal.Seq Length: 430 February 24, 1997 11:27 Type: N Check: 1785 ..

```
   1  ANCGAACATGG CCCCGCCCGC GCTCCANGCC CAGCCTCCAN GCGGCTCTCA
  51  ACTGAAGGTTC CTNCTGTTCC TGCTGCTGTT GCTGCTGCTG CTGTCATGGC
 101  CATCGCANGG GGNCNCCCTG GCAATGCCTG AACAGCGACC CTCCGGCCCT
 151  GAGTCCCAAC TCNACGCCGA CNAACTACGG GGTCNCTTCC AGGACCTGCT
 201  GAGCCGGCTG CATGCCANNC ATAGCCGAGA GGACTCTAAC TCTGAACCAA
 251  GTCCTGACCA TCTGTCNGGA TACTCANTCC ACANGTGAGA TTGGGGTCCA
 301  CGGCCAGCTG CTNCTCCNCG TCAACCGGGC GTCGCTGANT CANGGTCTCC
 351  CCGAACCTAC CNNGTGCANC NAGCGCTGCT CCTGCTGACN NCNAANGCCG
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SEQ ID. No.: 17

FIGURE 24

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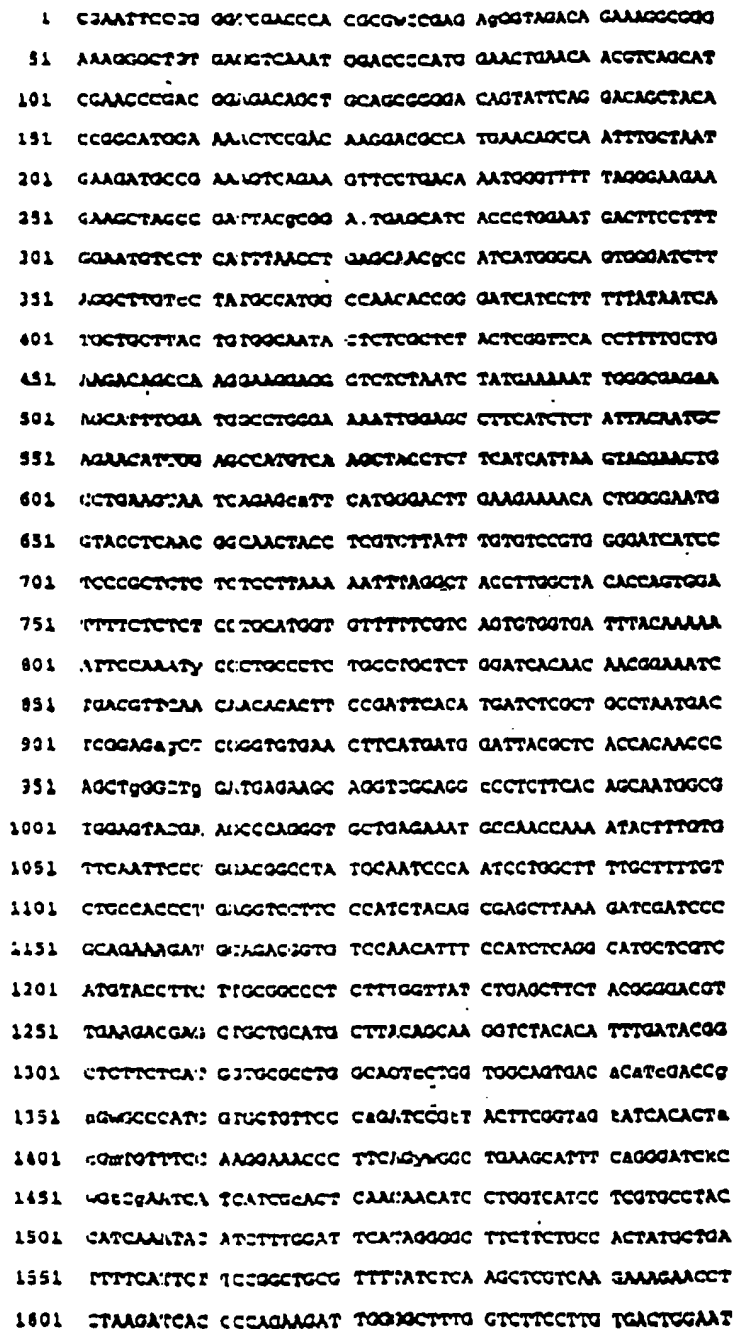
D77 - TA64/pEY

```
  1 CGGACGCGTG GCGAAAGCGG GTGGGGATAT ACGTGTGCTG GTGCCCTACA
 51 ATTCGACAGG CGTCTTCGGA GGGTCGACCA CCTTGCACTG TAGTCTGACT
101 TCTAATGAGA ATGTGACTAT CACTCAAATA ACCTGGATGA AGAANGATTC
151 AGGTGGACCC CACGCTCTTG TGGCTGTCTT CCACCCCAAG AAGGGCCCA
201 ACATCAAAGA GCCAGAGAGG GTGAAATTCT TGGCTGCCCA ACAGGATCTG
251 AGGAACGCAT CTCTGGCCAT CTCGAACTTA AGTGTTNAAG ACGAANGCAT
301 CTATGAACGT CNGATTGCCA CATTCCCCAG AAGCAGTANA AGCACCAATG
351 GCTGGCTGAA NGTGCAAGCC CGACCTNANA ACACTGCANA AGCCCTGGAC
```

SEQ ID. No.: 18

FIGURE 24



SEQ ID. No: 19

FIGURE 24

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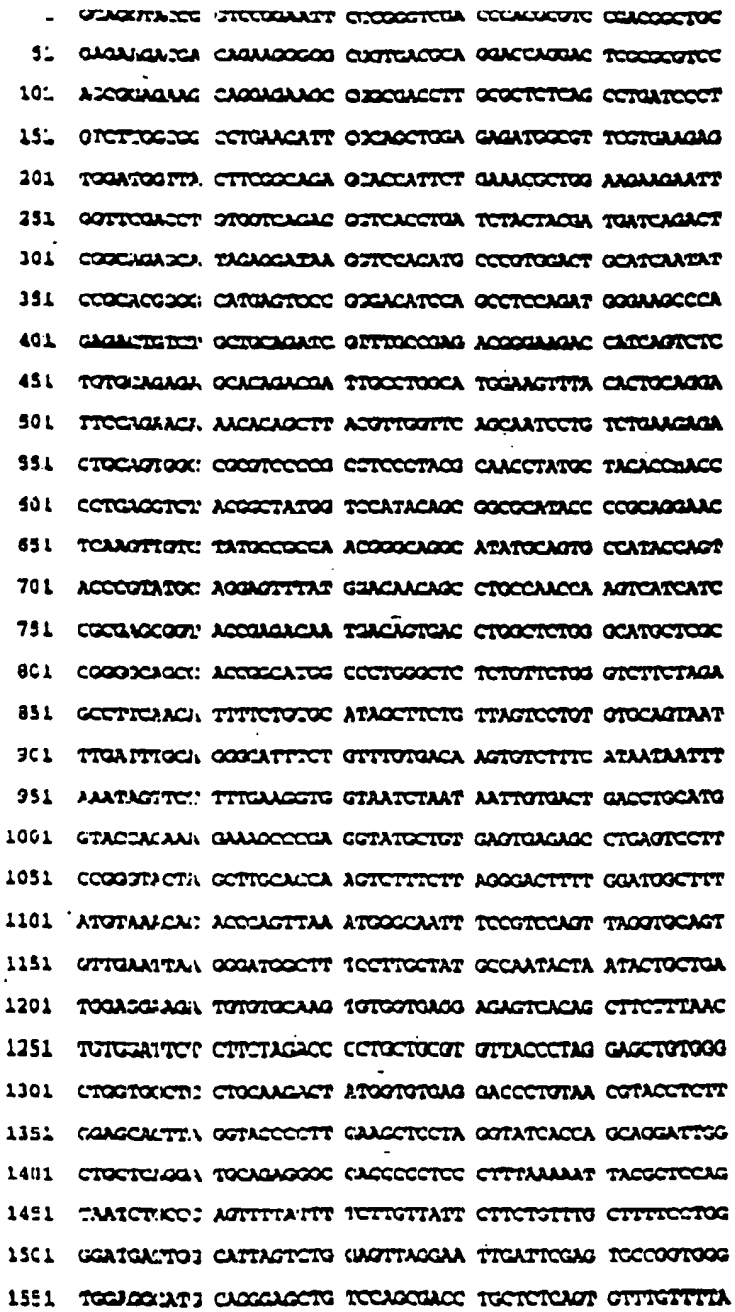
AFTP 1

1651 TATTTTCATG ATGGGAAGCA TGGCGCTCAT TATACTCGAC TCGATCTACA
1701 ACCCGCCGAA TCCCCAATCAC CACTAATCCC GCGGAGACGC GTCTCCACTG
1751 GAAACAGCTG AAATTGTCTG AAGGACATTT TAGTTGTCTT GATTGGCATG
1801 TTAGTCTGAG GAATTAGCAA GATTCCAAAG ACGTTTTTCT AGCTCTATCA
1851 GCACACATTT TAACCCAGCC CGTGCAGTGC AGTGTGTGAT GCCCGAGTTG
1901 TGTTTCGCAGC AGCTGTGCAA GCTGAAGCCT GTTGGCTGCG TGTGTTGGTC
1951 AGCAGACAAT AGCCTGTCCC CCCATGGTCA CTCCACTTCT CTCCACCCCC
2001 AGATTAACAG GTAATTCTAC TCTCAGAACA TCAGACAAAG ACCTCCTGGT
2051 TGGGATACTT GTCGAAGAGA AAATTATGGG TTTTGTTGGG AATGGTTTTG
2101 TTTGGCAATGG TGAAGGATGC ATTAAAAATT CTGTGGCACA CATTTTAACC
2151 CAGGCCGTGC AGTGCAGTGT TTGATGCCCG AGTTGTGTTT GCAGCAGCTG
2201 CGCAAGCTGA AGCCTGTTGG CTGCGTGTGT TGGTCAGCAG ACAATAGCCT
2251 GTGCCCCCAT GGTCACTCCA CTTCTCTCCA CCCCCAGATT AACAGGTAAT
2301 TCTACTCTCA GAACATCAGA CAAAGACCTC CTGGTTGGCG AAGTATCATC
2351 AGTTACGGCC ATCTCTCACT CTACACCAAC ACTAAGGGTC GGTTGACTAG
2401 CTGAGCCAGG GCGATATCTT GGGCTGTCCC TGTGAGGATC ATGACGTATG
2451 ACGGTTGCCA GTATAGAGTA CTTCATTTCA ATACTCAAGG AATAGTTTGC
2501 CCAACCTACT TATTACACCG AGTTAGTGAA

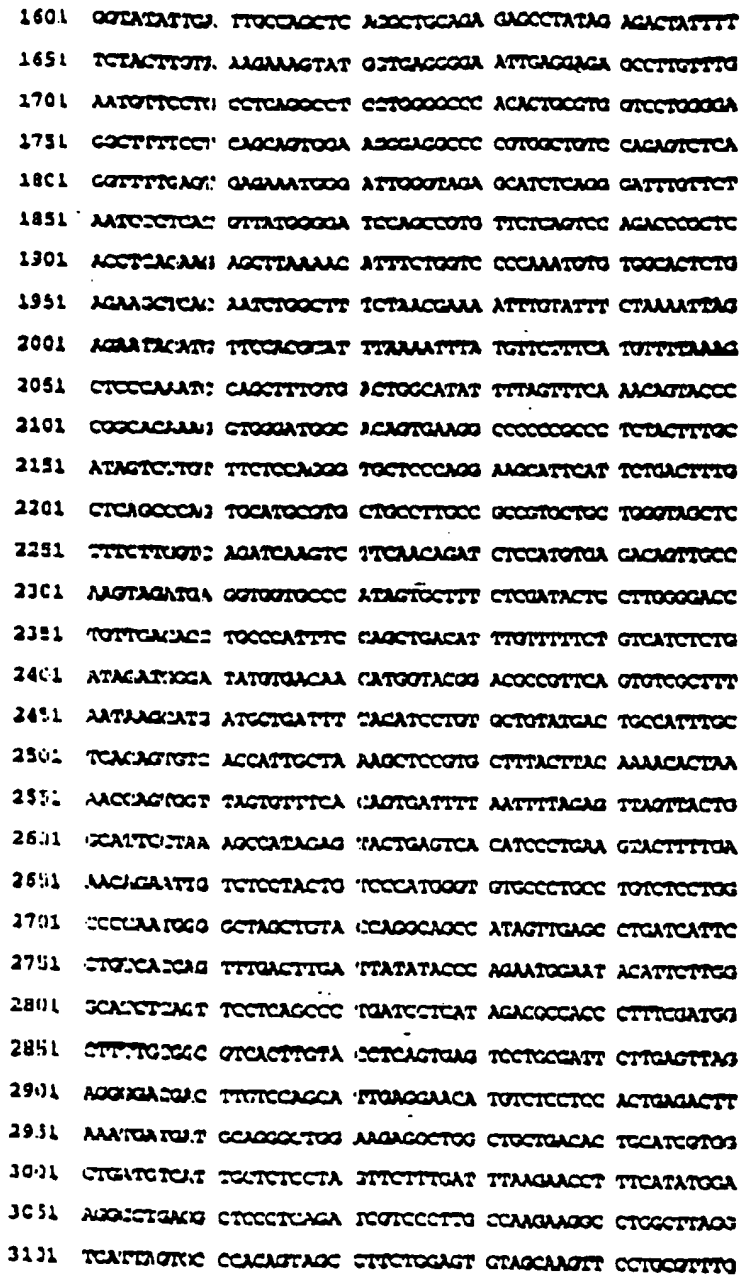
SEQ ID. No: 19 (cont'd)

FIGURE 24



SEQ ID. No: 20

FIGURE 24



SEQ ID. No: 20 (cont'd)

FIGURE 24

DD136

Session Name: crick.princeton.edu 1 Page 3

3151 AGACAGAATG GTTCAGATTT ATTTTCTACA TCTGTTGTTG ACCCCATGCA

3201 CCCTCTCATT TTGCCTTCCA GTCTACGTAG ATGAAAGATG AAAGGCAGAG

3251 GATGCAGACG GTCTTCTTTG TAATTGCTTC TGTTATTCTG TTGCATCTAC

3301 CGAGCCCGTT TTCTCCCTGT CTGTGCATAC AGTATGTTTA TAAGTGAACT

3351 TGTTAAAATA TTAAATGATC ACTAACCCTA AAAAAAAAAA AAAAAAAAAA

3401 AAAAAAAA

SEQ ID. No: 20 (cont'd)

FIGURE 24

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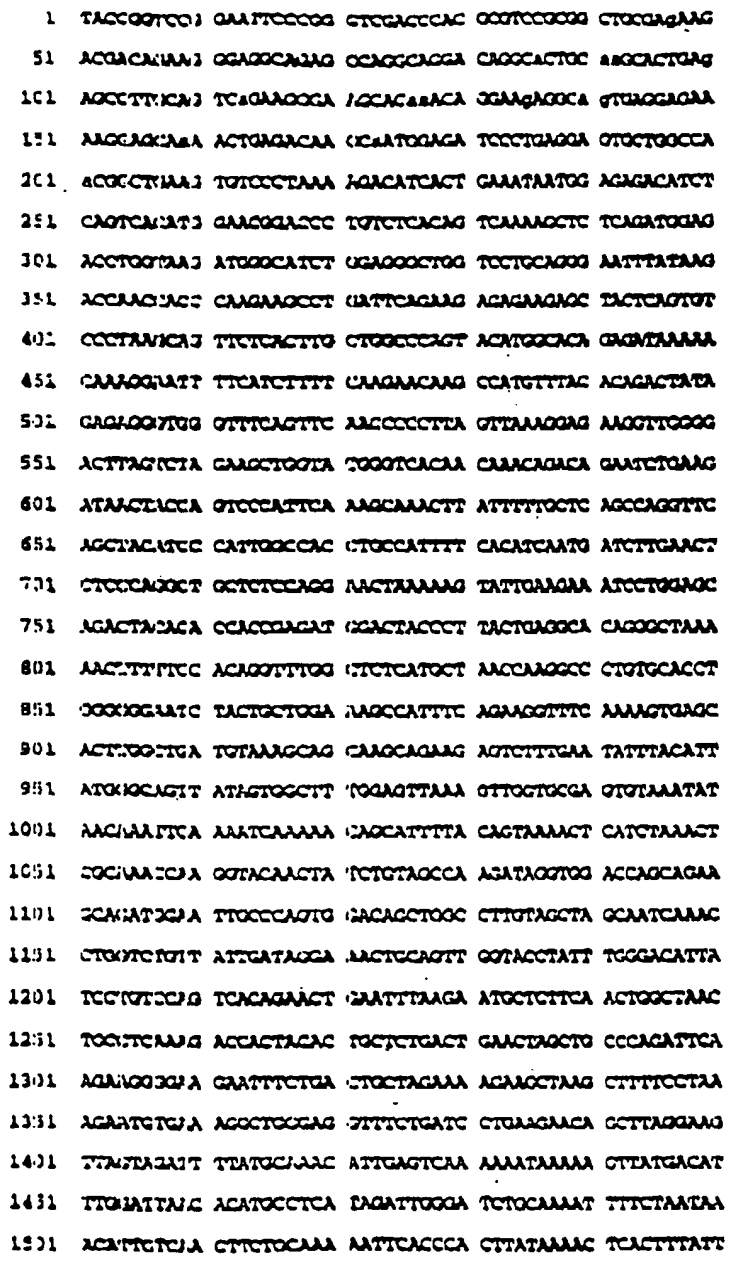

SEQ ID. No: 21

FIGURE 24

51/99

1551 AAATCTCAGT TGTGCAGCCT TCTAGAACCT CATGTCTACA AAGTGACAAA
1601 CTTTCCTCAA GCACAATCCA TCATACAGTG GATCAATCAG TCAGAGTCAG
1651 AGGAAGAACT AGTCAAAAAT AGCTCATTTT CTGAATTCAT TAACAGCTTA
1701 AAGAAAACCC ACAAATACCT AATGGAAGAG AGTTTCAAAA CTGAGCCCCC
1751 AGAAAGAGTG GAAGAAGCAA AGAGAATGGC TACATATGAA GTCACCACAG
1801 CTCTCAGCTC CTTCTTGAAG TACCTCAGAG AAACACAGCA GCCAGACATG
1851 CAGCTGCTCC TACTCTCCAT TGCTACTGGT GTAGGCTATC AGTTGGTAAA
1901 CAGTATTTTT CAGCATCTTC TGGGGTGTGA TGAGTTAAAC TTCCTCTTGG
1951 ATCAAACTGA AAATAACGAA CATAAATACC AAGAACTGAA AAATATTTGC
2001 AATTACAGAA CCCAGGCAGT CTTGGTGCTC ACAGCCCTAA GAGCCAGAGT
2051 TGAAATCACA GATGTTTCTA CAGAAGAGAA AGGACAACGT TTGACATTAA
2101 TACAACAACA TATGGGGTCA CTGTTGTCTG AAGAAGTTGC ACATGTCCTC
2151 ACAAAACATG GAGAACATCA TGACTGGGAA AGGCTGGAGA ATGATTTGAG
2201 ATTTGCTCAT GAGGGGGACT ATAAAGCCAC CACCCATTCC TTACAAATGG
2251 ATGAAGTAAA AAAACAATTG CAAAGTTTnT GCCATGAAAA GAAACAGACT
2301 TATAAACAAC AAGGTAATGA AAACAGAACA AAAGAAATGA TAGAAAATGG
2351 ACATTTCCTG GACTTACTCC AACGTTTAGG CCTAGACAAT TACTATCCAA
2401 AAAAAAAAAA AAAAAAAAAA AAAAAAAA

SEQ ID. No: 21 (cont'd)

FIGURE 24

52/99

Seqt/LL2-[redacted]

Session Name: crick.princeton.edu 1


```
  1 ACTAGACTCT CGTAGAGAAA AAAGTCCCTC GGAGTGGCTG CTGAACAACC
 51 TCATGACTCA CCAGAGTGTG GAGCTTTTCA NAAAAACTCA CTTNTAAACG
101 TCAGGAAACA GCTCATTCGT TCNTCTAACT TATTTGTGAT GCAAGTGGAA
151 ATAGATGTNT ATACAGCTCT TAAAAAGTGG ATGTTCCTTC AGCTGGTGCC
201 NTCCTGGAAT GGGGTCTTTG AAAACAGCTT TTGACAAAAA CAGATGTCTG
251 GTTTTCCAAC TGGAAAGAAG ACTTTGAAAG GACGACTTTC CNTGAAACTG
301 AACAGGGAAA ACCAGNTGGC GCCCNTGTNC CGACATTTAA GGCTACAGTA
351 CATTATCAGT GATCTGGCTT CTGCAAGGAT CATTGAGCAG GATTCTCTGG
401 TACCTTCAGA ATGGCTGGCG GCAGTGTATA AACAGCAGTG GCTGGCTATG
451 CTACGGCTG AACAAGACAG TCAAGTGGCG CCTCAAGAAA TCAATAAAGA
501 AGAACTTGAC GGAAACAGCA TCAGGTGTGG TCGAAAGCTT GCCAAAGATG
551 GTGANTACTG CTGGCGCTGG ACACGCTTCA ATTTCGGCTT TGACCTCCTT
601 GTGACTTACA CCAATCGATA CATCATTTTC CAACGCCATA CNCTGAACCA
651 GCCATGTTAT TGGATCTGTC AGCTTACACC TCGAAGGAGC ATAGCATTTA
701 AATGCGCTTT GCTTCTTTGA CAGTAGTGGG A
```

SEQ ID. No: 22

FIGURE 24

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Session Name: crick.princeton.edu 1

LL2-35(gd)

```
   1  GACATTTLAG GCTACAGTAC ATTATCAGTG ATCTGGCTTC TGCAAGGATC
  51  ATTGNGCAGG ATTCTCTGGT ACCTTCAGAA TGGCTGGCGG CAGTGTATAA
 101  ACAGCAGTGG CTGGCTATGC TACGGGCTGA ACAAGACAGT GAAGTGGGGC
 151  CTCAAGAAAT CAATAAAGAA GAACTTGAGG GAAACAGCAT CAGGTGTGGT
 201  CGAAAGCTTG CCAAAGATGG TAAGTACTGC TGGCGCTGGA CACGCTTCAA
 251  TTTCGGCTTT GACCTCCTTG TAACTTACAC CAATCGATAC ATCATTTTCC
 301  AACGCCATAC NCTGAACCAG CCATGTTATT GGATCTGTCA GCTTAGACCT
 351  CGAAGGANCA TANCATTTAA ATGCCCTTGG CTTCTTTGAC AGTAGTGGGA
```

SEQ ID. No: 23

FIGURE 24

54/99

Unix - tape

Session Name: crick.princeton.edu 1


```
   1 TAAGTGCAAC GCAATTAATG TAAGTTAGCT CATTCATTAG GCACCCCAGG
  51 CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT TGTGAGCGGA
 101 TAACAATTTC ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCTA
 151 ATACGACTCA CTATAGGGAA AGCTGGTACG CCTGCAGGTA CCGGTCCGGA
 201 ATTCCCGGGT CGACCCACGC GTCCGACGGC TCCTAGAAGA CGACAGAAGG
 251 GGGCACCACA GAGGACCCCT TCAACCTAAG GAAACACCCA GGCTTCGATA
 301 GGACCATGCT GCAGAGGTGG CAGAAAAGGG AGATCAGCAA CTTTGAATAC
 351 CTCATGTACC TCAACACACT GGCCGGAAGG ACCTACAATG ACTACATGCA
 401 GTATCCCGTT TTTCCCTGGG TCCTCGCTGA CTACACCTCA GAGATGTTGA
 451 ACTTTACGAA TCCCAAGACT TTCCCGGATC TTTCTAAGCC AATGGGGGCT
 501 CAGACCAAGT AAAGGAAGTT GAAGTTTACC CAGAGGTTTA AAGATGTTGA
 551 AAAGATTGAA GGAGACATGA CCGTGCAGTG CCACTACTAC ACCCACTATT
 601 CCTCAGCCAA CATTGTCGCT TCCTACTTGG TCGAAATGCC ACCATTCACG
 651 CAGGCCTTCA GCTCCTTACA GGGCGAAGC TTTGATGTGG CTGATAGAAT
 701 GTTCCACAGT GTAAAGAGCA CGTGGGAGTC TGCCTCCAAA GAGAACATGA
 751 GCGATGTCAA GGAGCTGACA CCTGAATTCT TCTACCTGCC CGAGTTTTTA
 801 ACCAACTGTA ATGCAGTGGA gTTTGGCTGC ATGCAGGATG GAACGACACT
 851 GGGGGATGTG CAGCTTCCTC CCTGGGCTGA CGGGGATCCG AGGAAATTCA
 901 TCAGCTTGCA CAGACAGGCT CTGGAAAGTG ACTTCGTCAG CAGCAACCTC
 951 CACCACTGGA TAGACCTAAT TTTTGGGTAT AAGCAGCAGG GGCCGGCTGC
1001 TGTAGACGCA GTGAACACTT TCCACCCCTA CTTCTACGGT GATAGAATAG
1051 ACCTGGCGAA CATCACTGAC CCGCTGATCA AGAGCACCAT CCTGGGCTTC
1101 ATCAGCAACT TTGGACAGGT GCCCAAGCAG ATCTTCACTA AACCCCACCC
1151 ATCCAGAAAA ACCACAGGGA AAAACCCAGG GCCTGGAAAG GATGCTTCCA
1201 CCCCTGCAGA CCTCCCAGGC CACTCACAGT CCTTCCTCCA CAGCCTGCCA
1251 GCACTGAGAC CCTCTCAGGT CACAGTCAAA GATATGTACC TTTTCTCTCT
1301 AGGGTCAGAA TCCCCCAAAG GGGCCATCGG CCACATCGTC CCTACTGAGA
1351 AGTCAATCCT GGCAGTGGAG AAGAACAAGC TGCTGATGCC CCCTCTCTGG
1401 AACAGGACCT TCAGCTGGGG CTTTGATGAC TTCAGTTGCT GCCTGGGGAG
1451 CTACTGGCTC GACAAGATCC TGATGACCTT TGAGAACCTG GCTGCCTGGG
1501 GTCCCTGTCT GTGCGCTGTA TGCCCTTCCC CCACGATGAT CGTCACATCC
```

SEQ ID. No: 24

DDI-6-1a.pc

Session Name: crick.princeton.edu 1 Page 2

1551 GGGGCCAXCG CAGTGGTGTG CATCTGGGAG CTGAGCCTGG TCAAAGGTCG
1601 CCCGAGAXGT CTGAAACTCC GACAGGCCTT GTATGGACAC ACTCAGGCGG
1651 TCACATGTCT GACAGCCTCT GTCACCTTCA GCCTCCTGGT GAGCGGATCC
1701 CAGGATCXCA CTTGTATCCT GTGGGACCTG GACCACCTCT CTCGTGTGGC
1751 CTGCCTGCCT GTCCACCGGG AAGGCATCTC AGCCATTGGC ATCAGTGATG
1801 TCTGXGGAAC CATTGTCTCC TATGCCGGAG CCCACTTGTC CCTGTGGAAT
1851 GTCAATGGAC AGCCCCTGGC CAGTATTACC ACAGCCTGGG GCCCAGAAGG
1901 AACCATAACC TGCTGCTGCA TAGTAGAGGG GCCAGCGTGG GATGCAAGCC
1951 ACGTXATCAT CACGGGGAGT AAGGACGGAA TGGTTCGGAT TTGGAAGNCA
2001 GAGGACCTCA AGATGCCTGT TCCCAGGCAG GCAGTGATGG AGGAGCCCTC
2051 CACGGAGCCC CTAAGCCCCA GAGGTCACAA GTGGGCCAAG AATCTTGCCC
2101 TGAGCCCAGA GCTGGATGTC AGTGTTGCTC TGAGTGGCAA GCCCAGCAAG
2151 GCAAATCCTG CTGTGACAGC TCTGGCCATC ACTAGGAACC AGAGCAAGCT
2201 CTTGGTTGGC GATGAGAAGG GCGAATCTTC TGCTGGTCTG CTGATGGGTA
2251 GGAGACAAGA AGCTGGAGGG ACCGACCTGA AAGCCTGGAG CCCTGGGGTC
2301 GGCAGCAAGA GGCTACAGGC ACAAGATGAT GTGTAGCCTG GGCTGCTTAA
2351 CCAGAGCAAA TTTTGGGGGG GCTCCACTCC ACACAGTTCT CAAGGAGTCC
2401 CTGATGGTTT GCACCGTGTA CCCTAAACAT GTCTGTAGTC TATGGGACTT
2451 CTGTAACAAA GATCTTGGTA GACACTGATG CTGGAAACTG ACGCTGCATG
2501 GGAAATTTCT ACGTGGCTCA CTTCACCAAG GCTTATTGCA CTGGGAAAAG
2551 AAGAGGKTGT GGGTATTGGT TCATGGAAAC CACCCCACTG TCTTTATTTT
2601 ATTAAAACTC CATTTTCCCA AAAAAAAAAA AAAAAAAAAA AAAAAAAAA

SEQ ID. No: 24 (cont'd)

FIGURE 24

Session Name:crick.princeton.edu 2 Page 1

Hdd2.Seq Length: 499 August 12, 1997 14:21 Type: N Check: 4083 ..

```
   1 CCCATTACTT CCACCTCCCA TGAGGACTGC ACCTGCGGCA GCGATTTTAT
  51 GTGAACCTGT ATTACTGTAT GGAAATGGAG GAGTCAGAAA GTGTGGGAAT
 101 CATAAAAGAA GGCAGAACCA AAAGACAGAG GGATCTTGGG CCATGAGTCA
 151 CAGCAAAGAA AGCCTCCCAC CAAACATCTG CACTGGACTC CTACAGGAAC
 201 AAGAAACTGA CTATCGTGTT CAGCCCCTGA GACTTGGGCT TAACATGATT
 251 TTCACACTGT CTACCCTACC TAATAGACCA GAGATGTAAA TATTATTCTT
 301 ATTTTAAAAG TGTGATGCCT CAGCTGCAAT GGGTGAGAAC TACTCCTCAT
 351 TTATTCTCTT CCAAGGCAAT AAAAGAGAAT GGACCAAAGA CAGTCTGTCA
 401 TCACATCTAG TCAAAAGAGC CAATGTCGCA GTACAACTCT TCAAAAGAAA
 451 AAGAAAAAAC AAGAAAAAAG CAATAAACAG ATGTGTTCTG CTTGAAAAA
```

SEQ ID. No: 25

FIGURE 24

Hdd 2.

Session Name:crick.princeton.edu 2

14-A5-T7.Seq Length: 686 July 24, 1997 11:39 Type: N Check: 9884 ..

```
   1 CGGGGTCGCC CGGGAATTAA GTGAGTCCAT TCTTGCACAC CGAGAAAATA
  51 TTCGNCANAT GATAAGAAGT TTTTCTGAAC CCTTTGGGAA GAGACTTGCT
 101 CAGTNTCTCT GATGGTAGAN GTAGAGCTCA TAATCGTAGA GGACATAATG
 151 ATGGCGAAGA TTCTTTGACT CATACAGATG TCAGCTCTTT CCAGACAATG
 201 GACCAAATGG TGTCTAATAT GAGAAACTAT ATGCAGAAAT TAGAAAGAAA
 251 CTTCNGGTCAA CTTTCAGTGG ATCCAAATGG ACATTCATTT TGTTCTTCCT
 301 CAGTTATTAC TTATTCCAAA ATAGGAGATG AACCGCCAAA GGTTTTTCAT
 351 GCCTCAACTC AAACTCGTCG AGCTCCAGGA AGAATAAAGG AAACCAGGAA
 401 AGCNATGAGA GATTCTGACA GTGGGACTAG AAAAAATGGC TATTGGTCNT
 451 CATATCCATG AACCGAGCTC NTGTTCNTTA ATAAAGTCCN NNGANCCNAG
 501 AAAGACTGGC AGATGAAAAA AGGTCCACCC AGGAANTTCA TCCCTTATGA
 551 AATGAAAATT GATGCCCCCT GCCTTTTGAA TGAAGGAATT GGCCAAATTN
 601 AAGGTTTTTC AAATTTTCAA ACCCCCGAAC AACCCAATCC TNGGAAAACC
 651 CCTNGAAATC AAAAAATTTT TTGGGCCCTT AAAAN
```

1 (3;5) (q25.1; q34; ... gene, NPM-MLF1

SEQ ID. No: 26

FIGURE 24

34-07.Seq Length: 651 July 24, 1997 11:37 Type: N Check: 6226 ..

```
  1 CAAAAN@ACI TGTGTTTAGC AAATAAAAAC TGCCCAGTGG GACAANCGTC
 51 GCCGGGAATC GCTGTCAGTA CTGCCGANTT CANAAATGCC TGGCTGTTGG
101 GANGGNCAAA GAAATGGNNC GCACAGACAG NNNAAAAGGC CGGAGAAGNC
151 GNNTGCCCCN GAAACCGAAG AACCACANGA NCCCTCTCCC CCTTCGCCCC
201 CGTTCCCNCC GAACCTGACN ANCAAANGAA NGGAGANGAN ACCCAGCANA
251 NCCNGCAANT CTATGANCTC CTGACTGGCT CCANGGAAAN CATCCCGGGC
301 NGGGCANAAA AAATCCCTGG GCNNCGCAGA ACTGCCCCAA GCCGACCAAG
351 AACTGCTNNC TGAANCAGCN NTCTNNGAAC TGNTTGTCCN NCCANNGCCA
401 NACAGAAGAT GAACATCGAC ATNTCTGCCT TCTCCTGCAT NGCNGCCCTG
451 GGCTATNGNC ACGNAAAAAC ACGGCNCNGG GAACCGGAAA ANGGAANAAN
501 GNCNNCGCNA TNGNTGANTC NCGCGGAGAC ACGNGACTTT CNACNANGGG
551 GGGGCGTANC GCCCCCCTTN NTTGTTCGNA CTGGGGGGGG GAACNNCCCN
601 AAATTCGNGC CCCTNNGGGC CGNAGGGGGG GGGGGGNCCN TTTTCTTTCT
651 N
```

TINUR = NGFI-B/nur77 beta type transcription factor

SEQ ID. No: 27

FIGURE 24

Session Name:crick.princeton.edu 2 Page 1

34-P4.Seq Length: 683 July 24, 1997 11:37 Type: N Check: 1900 ..

```
   1 GGGTCTGTTT GGACTGCAGA AGCAAGGGGG TGATGTAGCC CATCCTTCCC
  51 TTTGCAGATG CTGAGGGTGT TTCTTCCTGC ACCCACAGCC AGGGGGATGC
 101 CACTTCTTCC TCCGGCTTGA CCTGTTTCTC TGCCGCTACC TCCCTCCCCG
 151 TCTCATTTCG TTGTCTGTGG ATGGTCATTG CAGTTTAAGA GCAGAACAGA
 201 TCTTTCTATT TGGCCGCTTG AAAAGCTAGT GTACCTCCTC TCAGTGTTTT
 251 GGACTCCATC TCTCNTCCTC CANTACCTTG CTTCTTACTG ATAATTTTGC
 301 TGGAATTCCT AACTTTTCAA TGACATTTTT TTTAACTACT ATATTGATTG
 351 TCCTCTTAAA AAGAAAAGTG CATATTTATC CAAAATGTGT ATTTCTTATA
 401 CGCTTTTCTT TGTTATACCA TTTCCTCAGC TTATCTGTTT TATATTTGTA
 451 GGAGAAACTC CCATGTTATG GAATCCCACT GTATGATTTA TAAACAGACA
 501 ATATGTNGAG TGCCTTTTGC AAGAAAAGG GTGTGTTTGA AATCATCCGG
 551 ANTCNGCCAN GAAACTGTCT CCAAAGGAAA CNCCNACCTC TCTGTTCCTT
 601 GCTGTTNTGC TGATCATCCG CCANAAGGTG CTTCCCCCCG AATTTTGGTT
 651 TGGTTNTGTT TNCCGAAAAA TTTTTTCCGN TTN
```

DAP-kinase

SEQ ID. No: 28

FIGURE 24

Session Name:crick.princeton.edu 2 Page 1

38-A5-T7.Seq Length: 624 July 24, 1997 11:37 Type: N Check: 1627 ..

```
   1  AATGCACCTC CTTGTATTCC CACTTTCGTA NTCATTTCGG TTCTGATCTT
  51  GTCAAACCCA GCCTGACCGC TTCTGACGCC GGGATGGCCT CGTTACTANA
 101  CTTTTCTTTT TAAGGAAAGG CTGTTTTTTT TTGANGGTTT TCAAAACATT
 151  TTGAAAAGCA TTTACTTTTT TGACCACGAN CCATGANTTT TCAAAAAAAT
 201  CCGGGGTTGT GTGGGTTTTT GGTTTTTGTT TTAATTTTTG GTTGCGTTGC
 251  CTTTTTTTTT TAATGGGGTT GGCCCCATGA AATGGGTGCC CCACTCACTT
 301  CTCTGAAATC GAACNGACTG TGAATCCGCT CTTTGTCNGA ANCTGAACAA
 351  NCTGTGCCTT TTTTCCAACT CCGTGTGACN TTTCTGAATG TTATGTGGTA
 401  AGACCCCGGC GGTGTGGCAN CAACTGCCCT GGAACCCCAN CCCCTGCNTC
 451  CATCTGTNGC TGNTGCGCCC CACANTAAAA CNTTCANACN TCCCTGAAAA
 501  GTTCTTTAAA AAANTTAATT ANATTGTCCC NGTTTTACTG GGAAAAAATT
 551  ANCCNTACTC CNGCCAATTT TNTTCTTNCN ANTGGGCTGA AAGAAATCCT
 601  TGTTCCCCAN TTTTCCTTTG GGCN
```

glycine-rich RNA binding protein

SEQ ID. No: 29

FIGURE 24

Session Name:crick..princeton.edu 2 Page 1

38-02.Seq Length: 641 July 24, 1997 11:37 Type: N Check: 656 ..

```
   1 ATCCCAGACC AGGCAACAAG GTCCTTTGAG GTCTATAATG AAAGATCTGC
  51 ATTCTGATGA CAATGACGAG GAATCAGATG AAGTGGAGGA TAACGACAAT
 101 GACTCTGAAA TGGAGAGCCC TCTTNAATAGA GCANGCAGCC GAAGTCGCAG
 151 AGTTAGCTTA AGTGATGGCA GCGATAGTGA AAGCAGTTCT GCTTCTTCAC
 201 CCCTCATCA CGAACCTCCA CCACCCTTAC TAAAAACCAA CAACAACCAG
 251 ATTCTTGNAG TGAAAAGTCC NATAAAGCAA AGCAAATCAG ATAAGCAAAT
 301 AAAGAATNGT GAATGTGACA AGGCATACCT AGATGAACTG GTNGAGCTTC
 351 CCRGAANNTT NETCACATTG AGAGAAAGAC ACATTCTGCA GCAGATCGTG
 401 AACCTTANNG AAGAAACTGG GACACTTTCA TATCACCAAC ACAACATTTG
 451 ATTTTGATCT TTGCTCGCTG GACAAAAGCA CAGTCCGTTA ACTACAGAGT
 501 TCCTNGAAAC ATCTGGAACA TTCTGAAGAT ATAACCACTG GATGCCTCNA
 551 GAACTAATGT GTTTNNNNNN NNNNNGGTT NNNNNNTTTG GGTGTGAATT
 601 TTTGTTCCCN TTGTTTAAAT GAAAACCCCC CNAATGATGC N
```

LTG9/MLLT3

SEQ ID. No: 30

FIGURE 24

LL5705

Session Name: crick.princeton.edu 1

```
  1 GCGAGCCGAC GGAGNGGCTC TACGGTGGAT AGCGTGTTCC GGAACCTATC
 51 CCTCGAATTA GCCGAGTCAG GCAGAGAGGG GGCGGGGNGT GCTTCCGCCC
101 TTGCTAGGAG GGGCTGCATT GCAUGGGAGA CCCAGCGGCA GATTCTGTCA
151 CAGACGAGGG AGAAGGCGTG AGGAGACAAA GCCGTCACAT CCGCGACAGC
201 TTCCTTCAGC AGCCTCCTCC TCTCCAGTCC AGAGCCGACC CCCGAGCCCC
251 TGAGGCATCC CTTCCGTCTT CGGAACACCC TAGTCATTCA TTGCTAACAG
301 GGGATCATGA GGGACCCTGT AAGTAGCCAG TACAGCTCCT TTCTTTTCTG
351 GANGATGCCC ATCCCACAAC TGGATCTGTC NGAGCTGGAA GCCCTGGGCC
```

SEQ ID NO: 31

FIGURE 24

63/99

Session Name: crick.princeton.edu 1

82-67


```
   1  ACGGCTGCGA GAAGACGACA GAAGNGAAGC TAAGGGCTGG TCGCTGCGTC
  51  TGAACGCCGG GTTGGACGAA CCGCTGTGCG CCCTTGGCGG ACGTNAGCGG
 101  AAAGAAGATG GCGGTGCAGG TGGTGCAAGC TGTGCANGCG GTTCATCTTG
 151  ANTCTGACGC TTTCCTANTT TGTCTCAACC ATGCTCTGAG CACAGAAAAN
 201  GANGAAGTGA TGGGTCTGTG TATAGGGGAN TTGAATGATG ACATAAGGAG
 251  TGACTCCNAA TTTACATACA CTGGAACGGA AATGCGCACA GTCCNGGAAA
 301  AGATGGATAC CATCAGAATT GTTCATATCC ATTCTGTCAT CATCTTGCGG
 351  CGTTCTGACA AGANAAAANA CCGTGTTNAA ATTTCTCCAN ANCANCTGTC
```

SEQ ID. No: 32

FIGURE 24

475

LL2-76

Session Name: crick.princeton.edu 1 Page 1

```
   1  GATGGCGGTC TCCACAGGAG TTAAAGTTCC TCGTAATTTT CGCTTGTTGG
  51  AAGAACTTGA AGAAGGACAA AAAGGAGTAG GTGATGGTAC TGTTAGCTGG
 101  GGCCTTGAAG ATGATGAAGA CATGACACTT ACAAGGTGGA CAGGCATGAT
 151  TATTGGGCCA CCAAGGACAA ACTATGAAAA CAGAATATAT AGCCTGAAAG
 201  TAGAATGTGG ATCTAAATAC CCAGAAGCTC CTCCATCAGT TAGATTTGTA
 251  ACAAAAATTA ATATGAATGG GATCAATAAT TCCAGTGGAA TGGTGGATGC
 301  ACGGAGCATA CCAGTATTAG CAAAATGGCA AAATTCCTAT AGCATTAAAG
 351  TCATACTTCA AGANCTAAGA CTCTTATGAT GTCCAAAGAA AATATGAANC
```

SEQ ID. No: 33

FIGURE 24

76

B3-77

Session Name: crick.princeton.edu 1


```
   1  ACGGCTGCGA GAAGACGACA GAAGGGGGAG CTTCTGCGTC CCAGGGACTC
  51  CAGTACACCA CCATGGCGGA TTCTGAGCGT CTCTCGGCCC CCGGCTGCTG
 101  GTTAGCCTGC ACCAGCTTCT CGCGCACCAA AAAGGGAATT CTCCTGTTTG
 151  CTGAAAATTAT ACTGTGCCTG GTGATCTTGA TTTGCTTCAG TGCATCTACA
 201  ACATCGGCCT ACTCCTCCCT GTCGGTGATT GAGATGATCT GTGCTGCTGT
 251  CTTACTTGTC TTCTACACGT GTGACCTGCA CTCCAAGATA TCATTCATCA
 301  ACTGGCCTTG GACTGACTTC TTCAGATCCC TCATAACAAC CATCCTGTAC
 351  CTGATCACCT CCATTGTTGT CCTTGTAGAA AGAAGAAGCA GCTCCAGAGT
```

SEQ ID. No: - 34

FIGURE 24

77

Session Name: crick.princeton.edu 1 C2-48


```
   1  TACGGCTGCG AGAAGACGAC AGAAGGGGCT CCGCCTTCTT CCCCACCTCC
  51  GGCTGCCGGC GACACCGGTC TTTGCAGTCC GGGACCCCGT GCGATCGTTA
 101  GCCCCGTGCA CGATGCCCCC GAAAAAGGGA GGAGATGGAA TTAAACCGCC
 151  TCCAATTATT GGAAGATTTG GAACCTCACT GAAAATTGGT ATCGTTGGAT
 201  TGCCAAATGT TGGGAAATCT ACCTTCTTCA ATGTATTAAC CAATAGTCAG
 251  GCTTCANCAG AAAACTTCCC ATTCTGCACT ATTGATCCTA ATGAGAGCAG
 301  AGTGCCTGTG CCAGATGAGA GGTTCGACTT TCTTTGCCAG TACCATAAAC
 351  CANCAAGCAA GATCCTGCTT TCCTAAATGT AATGGATATT GCTGGCCTTG
```

SEQ ID. No: 35

FIGURE 24

C325

Session Name: crick.princeton.edu 1


```
  1  ACGGCTGCGA GAAGACGACN GAAGGGGTCC TTTTTCCTGC GCGGCGTTTC
 51  AGTCCCTCTT GAGTTGGGCT GTCGTCNANT CGCTGCCGAN CCGGCGCACC
101  GCAGCCATGA CAGAAGCTGA TGTNAATCCG AANGCCTATC CCCTCNCANA
151  TNCCCACCTC ACCAAGAANC TGCTGGACCT TGTTCNACAG TCTTGTTNCT
201  ACAANCAGCT TCNGAAAGGA CCNATGAACC NCCAAAACCC TCAACAGAAG
251  CATCTCTGAN TTCATTGTGA TGCNGCANAN CTGAACCTTG GAGATCTCCT
301  GCACCTCCCT CTNCTGTCGA AAAAAANAAT GTCCCTACCT NTTTGTNCNN
351  CCAACAGGCT TTANGAAAGG CCTNTGGGTT TCCAGCCNTC ATCCCTGTCT
```

SEQ ID. No: 36

FIGURE 24

50

LL2-89

Session Name: crick.prin_eton.edu 1


```
   1  GAAAGGCGCC TAGATACCGC GATACTTGCG GGTATCCTGG AGCTGGACTC
  51  TCCTGTTCTT GCCACATTTG ACTGAAGAAG GACAACGGGG TCAAGGTTTG
 101  AGGACTGACA GATTCTANAC CCAGGCTTCC TCNGGCCCCA NAAAGCCACC
 151  ANACCTCACA TGGAACCAAA GGCCCCATGC CCANCCGCCG TCCCCTCAGA
 201  GGAANANGAAA TTCCGTGTTC TTGTNGGCGT CACTGGCAGC CTGGCCGCTC
 251  TGAAGCTGCC TCTCCTGNTA TCTANNCTGT TGGACGTTCC TGNGATGTGG
 301  AAGCGCCGTT CTGACCCGGT TCTCCACATT GACCTGCGGA TGTGGGCTGA
 351  CCTCATGCTA GTGGCTCCCC TCNATGCNNA CACTCTGNGG AAGT
```

SEQ ID. No: 37

FIGURE 24

51

Session Name: crick.princeton.edu 1 SBS-A56 Page 1

```
  1  ACGGCTGCGA GAAGACGACN GAANGGGGGC AGGATGGCTC GGTGGTACAG
 51  TTCAAGATCA ANANGCATAC CCCACTGAGC AAGCTGATGA AGCCTACTGT
101  GAGAGGCAGG GCTTGTCNAT NANGCAGATT CGATTCCGGT TTGATGGACA
151  ACCAATCAAT GAAACAGACA CTCCACCCCA NCTGGAGATG GAGGATGAGG
201  ACACCNTTGA TGTATTCCAG CAGCAGACAG GAGGATCANC CTCCCGAGGG
251  AGCGTCCCAC ACCCAACCGT TGTCCTGACC TGTGCTATTG AGCAGTGACC
301  ATGCTNCCAC ACCCA
```

SEQ ID. No: 38

FIGURE 24

To

C4-23

Session Name: crick.princeton.edu 1


```
  1  ACGGCTGCGA GAAGACGACA GAAGGGGAGA GCATCATGGC GCTAAGCGGT
 51  CGACTGGCAT TGGCCGCGCT CAGACTGTGG GGTCCGGGAG GATGTATCTG
101  ANCTGCTTCG GACCAAAACC TCCAGTAGGG TGGTGGTCAT GGTGGGGGCC
151  GGCATCAACA CACCCAGTGG CATCCCGGAC TTCAGATCCC CAGCGGAGCG
201  GCCTCTACAG CAACCTTCAG CAGTATGACA TCCCGTACCC TGAAGCCATC
251  TTTGAACTTG GCTTTTTCTT TCACAACCCC AAGCCCTTTT TCATGTTGGC
301  CAAGGACTGT NCCCTGNGCA CTACAGGCCA ATGTCACTCA CTACTTCCTG
351  AAGCTCCTCC ACGACANGGA CCGCTTCTGC GCTCTNTACA CANAAATCGA
```

SEQ ID. No: 39

FIGURE 24

52

LLS-68

Session Name: crick.princeton.edu 1

```
   1  GGCCATCAAN CGTGCTGTTG GTCATGGCCG ACTCCTCAAA GATGGACCCC
  51  TCTACCGCCT CAACACTAAG GCAGCCAGTG CTGAAGGGAA AGAGGGCTGT
 101  GAGTCCCTCT CGTGTTTACC TCCCGTGTCA CTGCTTCCAC ACGANAAAGA
 151  CAAGCCGGTT GCTGAACCAA TCCCCATCTG TAGCTTCTGT CTTGGTACAA
 201  AAGAACAGAA CCGGGAGAAG CAGCCCGAGG AACTCGTCTC CTGCCCGGAC
 251  TGCGGCAATA NCGGTCATCC ATCGTGTTTA AAGTTCTCCC CANAGCTNAC
 301  AGTGAGAGTG AAGCCTTACN GTGGCTTNCA TTGANTGTAA AACATCACT
```

SEQ ID. No: 40

FIGURE 24

>SCILL5-96-full.seq|14350
CCCACGCGTCCGATCTCCTCCAGGGCCACCAAGCACCTCTGAAGAGCCATGTTCCAAGCTGCCGGAGCCGCCCAGGCCAC
CCCCTCTCATGAAGCCAAAGGCAGCAGTGGCAGCAGCACGGTACAGCGGTCTAAGTCCTTTAGCTTGCGGGCTCAGGTGA
AGGAGACCTGTGCAGCCTGCCAGAAGACTGTGTACCCGATGGAGCGGCTGGTGGCAGACAAGCTCATTTTCCACAACTCT
TGTTTCTGTTGCAAACACTGCCACACCAAACTCAGCCTGGGCAGTTATGCTGCAATGCACGGTGAATTTTACTGCAGACC
TCACTTTCAGCAGCTGTTTAAGAGTAAAGGCAACTACGATGAAGGGTTTGGTCGTAAACAGCACAAGGAGCTCTGGGCCC
ACAAGGAGGTGGACTCAGGCACCAAGACGGCCTGAGACCCCTTTAACACCCATTCCCTCCCAGCACATGGCCTCCCGCTG
GGCAGTGGAAAGGAGATTAACCCGGGGGCGCGGGGTGGGAGAGGATGAGGCTCCCTCACACAGGTTTCAGGCATAAGGCT
CTGCTCCAGGATTCCTTACTTTTCCCATGGGAGGTTGGCGTTGGGAACCAGAATTGGAATTTTCACCATACTGTGTCCTT
TAGTCCACCTCATCTCACCCCACGGCTCCCTGGGAGGCCCACAAGCCCAGCTTCCATACTTAGGTGCTTTTCTCCAGCAA
GGAGTCAGCATGCCCTCCTCAGGGTCCCAAGCTCCCTCACTGCCACCTGGGCCTTGTGTACCCCCTTGTCTCCCCATCTA
CCTCTGCCCCTTAGCCTGGTAATGAGCCACAGAGACTGGAAGAGGGAGAGTGCCATNTACTGGGCCTCATAGATGCCACC
TCGCTGAGGGGGGAGGGCTGGGGAAGAGGCAAGACAGCCTGCAGCCTTCAGGGTCTGGGGGTCCCTTGCACCACAAAGCT
AAAGCTCTTGCTAGAGCCTCAGCTGACAGGGTCGGCAGTAGCTATGCTCCTCCATCTGTTGTGCTGTTCTGTTGTGATCA
ACCCTCTTTTAAAAACATTTAAACAGCAAAAAAAAAAAAAA

SEQ ID. No: 41

FIGURE 24

```
>SC|LL5-96-full.seq|14350 Frame 1
MFQAAGAAQATPSHEAKGSSGSSTVQRSKSFSLRAQVKETCAAC
QKTVYPMERLVADKLIFHNSCFCCKHCHTKLSLGSYAAMHGEFYCRPHFQQLFKSKGNYD
EGFGRKQHKELWAHKEVDSGTKTA*DPFNTHSLPAHGLPLGSGKEINPGARGGRG*
```

SEQ ID. No: 42

FIGURE 24

>SCISA7-full

AGCTCTAGTC CCCAGAGATG TCGCCACTAC TGCTGCTGCT GCTGTGCCTG
CTGCTGGGGA ATTTGGAGCC TGAGGAGGCC AAACTGATCC GTGTCCCTCT
TCAACGAATC CACCTTGGAC ACAGAATCTT AAACCCACTG AATGGATGGG
AACAGCTGGC AGAGCTTTCT AGGACCTCCA CCTCTGGTGG CAACCCCTCC
TTTGTGCCTC TCTCCAAGTT CATGAACACC CAGTATTTTG GAACTATTGG
TTTGGGAACG CCTCCTCAGA ATTTCACCGT TGTCTTTGAC ACGGGTTCTT
CCAACTTGTG GGTTCCGTCC ACGAGATGTC ATTTCTTCAG TTTGGCATGC
TGGTTTCACC ATCGCTTTAA TCCCAAGGCC TCCAGCTCCT TCAGGCCCAA
TGGGACCAAG TTTGCCATTC AGTATGGGAC CGGGCGGCTG AGCGGAATCC
TGAGCCAGGA CAATCTGACT ATCGGGGGGA TCCACGATGC TTTTGTGACA
TTTGGAGAGG CTCTGTGGGA GCCCAGCCTG ATCTTTGCTT TAGCCCACTT
TGATGGGATC CTGGGCCTCG GCTTCCCCAC TCTGGCTGTG GGCGGAGTTC
AGCCTCCGCT GGATGCGATG GTGGAGCAAG GGCTGCTGGA GAAACCCGTC
TTCTCCTTTT ACCTCAACAG GGATTCTGAA GGGTCTGATG GGGGAGAGCT
GGTCCTAGGG GGCTCAGACC CCGCTCACTA CGTACCTCCC CTCACCTTCA
TACCAGTCAC CATCCCTGCC TACTGGCAGG TCCACATGGA GAGTGTGAAG
GTCGGCACAG GGCTGAGCCT CTGTGCCCAG GGCTGCAGTG CCATCCTAGA
CACAGGCACA TCCCTCATCA CAGGACCTAG TGAGGAGATC CGGGCCTTGA
ATAAAGCCAT TGGGGGATAT CCCTTCCTGA ATGGGCAGTA CTTCATTCAG
TGTTCCAAGA CGCCAACGCT TCCCCCTGTC TCCTTCCACC TTGGTGGAGT
CTGGTTTAAC CTCACAGGCC AGGACTATGT CATCAAGATT CTTCAGAGCG
ATGTTGGCCT CTGCCTGTTG GGCTTCCAAG CCTTGGATAT CCCCAAGCCT
GCGGGACCCC TCTGGATCCT TGGGGACGTC TTTTTGGGGC CCTATGTGGC
TGTCTTTGAC CGTGGGGACA AAAACGTCGG CCCGCGCGTG GGACTGGCGC
GTGCTCAGTC TCGTTCAACA GACCGGGCAG AAAGAAGGAC TACGCAGGCG
CAGTTCTTCA AAAGACGCCC TGGTTAGGGT ACAAGCTCAC CGGGCCACAG
CAGCTATGCT TCTTTCCAAT TAAACAAACT AAAAAAAAAA AAAAAAAAAA
AAAAAAAAAA A

SEQ ID. No: 43

FIGURE 24

```
>SCISA7-full Frame 3
MSPLLLLLLC LLLGNLEPEE AKLIRVPLQR IHLGHRILNP LNGWEQLAEL
SRTSTSGGNP SFVPLSKFMN TQYFGTIGLG TPPQNFTVVF DTGSSNLWVP
STRCHFFSLA CWFHHRFNPK ASSSFRPNGT KFAIQYGTGR LSGILSQDNL
TIGGIHDAFV TFGEALWEPS LIFALAHFDG ILGLGFPTLA VGGVQPPLDA
MVEQGLLEKP VFSFYLNRDS EGSDGGELVL GGSDPAHYVP PLTFIPVTIP
AYWQVHMESV KVGTGLSLCA QGCSAILDTG TSLITGPSEE IRALNKAIGG
YPFLNGQYFI QCSKTPTLPP VSFHLGGVWF NLTGQDYVIK ILQSDVGLCL
LGFQALDIPK PAGPLWILGD VFLGPYVAVF DRGDKNVGPR VGLARAQSRS
TDRAERRTTQ AQFFKRRPG
```

SEQ ID. No: 44

FIGURE 24

>SCIDD136114044

CGGTGACGCAGGACCAGGACTCGCGCGTCCAGCGGAGAAGCAGGAGAAGCCGGCGACCTTGCGCTCTCAGCCTGATCCCT
GTCTTGGCGGCCTGAACATTCGCAGCTGGAGAGATGGCGTTCGTGAAGAGTGGATGGTTACTTCGGCAGAGCACCATTCT
GAAACGCTGGAAGAAGAATTGGTTCGACCTGTGGTCAGACGGTCACCTGATCTACTACGATGATCAGACTCGGCAGAGCA
TAGAGGATAAGGTCCACATGCCCGTGGACTGCATCAATATCCGCACGGGGCATGAGTGCCGGGACATCCAGCCTCCAGAT
GGGAAGCCCAGAGACTGTCTGCTGCAGATCGTTTGCCGAGACGGGAAGACCATCAGTCTCTGTGCAGAGAGCACAGACGA
TTGCCTGGCATGGAAGTTTACACTGCAGGATTCCAGAACAAACACAGCTTACGTTGGTTCAGCAATCCTGTCTGAAGAGA
CTGCAGTGGCCGCGTCCCCGCCTCCCTACGCAACCTATGCTACACCNACCCCTGAGGTCTACGGCTATGGTCCATACAGC
GGCGCATACCCCGCAGGAACTCAAGTTGTCTATGCCGCCAACGGGCAGGCATATGCAGTGCCATACCAGTACCCGTATGC
AGGAGTTTATGGACAACAGCCTGCCAACCAAGTCATCATCCGCGAGCGGTACCGAGACAATGACAGTGACCTGGCTCTGG
GCATGCTCGCCGGGGCAGCCACCGGCATGGCCCTGGGCTCTCTGTTCTGGGTCTTCTAGAGCCTTCAACATTTTCTGTGC
ATAGCTTCTGTTAGTCCTGTGTGCAGTAATTTGATTTGCAGGGCATTTCTGTTTGTGACAAGTGTCTTTCATAATAATTT
AAATAGTTCTTTTGAAGGTGGTAATCTAATAATTGTGACTGACCTGCATGGTACCACAAAGAAAGCCCGAGGTATGCTGT
GAGTGAGAGCCTGAGTCCTTCCGGGTACTAGCTTGCACCAAGTCTTTCTTAGGGACTTTTGGATGGCTTTATGTAAACAC
ACCCAGTTAAATGGGCAATTTCCGTCCAGTTAGGTGCAGTGTTGAATTAAGGGATGGCTTTCCTTGCTATGCCAATACTA
ATACTGCTGATGGAGGAAGATGTGTGCAAGTGTGGTGAGGAGAGTCACAGCTTCTTTAACTGTGGATTCTCTTCTAGACC
CCTGCTGCGTGTTACCCTAGGAGCTGTGGGCTGGTGGCTCCTGCAAGACTATGGTGTGAGGACCCTGTAACGTACCTCTT
GGAGCACTTAGGTACCCCTTGAAGCTCCTAGGTATCACCAGCAGGATTGGCTGCTCAGGATGCAGAGGGCCACCCCCTCC
CTTTAAAAATTACGCTCCAGTAATCTGCCCAGTTTTATTTTCTTGTTATTCTTCTGTTTGCTTTTCCTGGGGATGATTGG
CATTAGTCTGGAGTTAGGAATTGATTCGAGTGCCGGTGGGTGGAGGCATGCAGGGAGCTGTCCAGCGACCTGCTCTCAGT
GTTTGTTTTAGGTATATTGATTGCCAGCTCAGGCTGCAGAGAGCCTATAGAGACTATTTTTCTACTTGTAAAGAAAGTAT
GGTGAGGGGAATTGAGGAGAGCCTTGTTTGAATGTTCCTGCCTCAGGCCTCCTGGGGCCCACACTGCGTGGTCCTGGGGA
GGCTTTTCCTCAGCAGTGGAAGGGAGGCCCCGTGGCTGTCCAGAGTCTCAGGTTTTGAGTGAGAAATGGGATTGGGTAGA
GCATCTCAGGGATTTGTTCTAATCCCTCATGTTATGGGGATCCAGCCGTGTTCTCAGTCCAGACCCGCTCACCTCAGAAG
AGCTTAAAACATTTCTGGTCCCCAAATGTGTGGCACTCTGAGAAGCTCACAATCTGGCTTTCTAACGAAAATTTGTATTT
CTAAAATTAGAGAATACATGTTCCACGCATTTAAAATTTATGTTCTTTCATGTTTTAAAGCTCCCAAATCCAGCTTTGTG
ACTGGCATATTTTAGTTTCAAACAGTACCCCGGCACAAAGGTGGGATGGCACAGTGAAGGCCCCCCGCCCTCTACTTTGC
ATAGTCTTGTTTCTCCAGGGTGCTCCCAGGAAGCATTCATTCTGACTTTGCTCAGCCCAGTGCATGCGTGCTGCCTTGCC
GCCGTGCTGCTGGGTAGCTCTTTCTTGGTCAGATCAAGTCTTCAACAGATCTCCATGTGAGACAGTTGCCAAGTAGATGA
GGTGGTGCCCATAGTGCTTTCTCGATACTCCTTGGGGACCTGTTGACACCTGCCCATTTCCAGCTGACATTTGTTTTTCT
GTCATCTCTGATAGATGGGATATGTGACAACATGGTACGGACGCCGTTCAGTGTCGCTTTAATAAGCATGATGCTGATTT
TACATCCTGTGCTGTATGACTGCCATTTGCTCACAGTGTCACCATTGCTAAAGCTCCGTGCTTTACTTACAAAACACTAA
AACCAGTGGTTAGTGTTTCACAGTGATTTTAATTTTAGAGTTAGTTACTGGCATTCCTAAAGCCATAGAGTACTGAGTCA
CATCCCTGAAGTACTTTTGAAACAGAATTGTCTCCTACTGTCCCATGGGTGTGCCCTGCCTGTCTCCTGGCCCCAATGGG
GCTAGCTGTACCAGGCAGCCATAGTTGAGCCTGATCATTCCTGTCACCAGTTTGACTTGATTATATACCCAGAATGGAAT
ACATTCTTGGGCATCTCAGTTCCTCAGCCCTGATCCTCATAGACGCCACCCTTTCGATGGCTTTTGCGGCGTCACTTGTA
CCTCAGTGAGTCCTGCGATTCTTGAGTTAGAGGGGACGACTTGTCCAGCATTGAGGAACATGTCTCCTCCACTGAGACTT
AAATGATGATGCAGGGCTGGAAGAGGCTGGCTGCTGACACTGCATCGTGGCTGATGTCATTGCTCTCCTAGTTCTTTGAT
TTAAGAACCTTTCATATGGAAGGCCTGAGGCTCCCTCAGATCGTCCCTTGCCAAGAAGGCCTGGCTTAGGTCATTAGTGC
CCACAGTAGCCTTCTGGAGTGTAGCAAGTTCCTGCGTTTGAGACAGAATGGTTCAGATTTATTTTCTACATCTGTTGTTG
ACCCCATGCACCCTCTCATTTTGCCTTCCAGTCTACGTAGATGAAAGATGAAAGGCAGAGGATGCAGACAGTCTTCTTTG
TGATTGCTTCTGTTATTCTGTTGCATCTACCGAGCCCGTTTTCTCCCTGTCTGTGCATACAGTATGTTTATAAGTGAACT
TGTTAAAATATTAAATGATCACTAACCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID. No: 45

FIGURE 24

>SC|DD136|14044 Frame 3
MAFVKSGWLLRQSTILKRWKKNW
FDLWSDGHLIYYDDQTRQSIEDKVHMPVDCINIRTGHECRDIQPPDGKPRDCLLQIVCRD
GKTISLCAESTDDCLAWKFTLQDSRTNTAYVGSAILSEETAVAASPPPYATYATXTPEVY
GYGPYSGAYPAGTQVVYAANGQAYAVPYQYPYAGVYGQQPANQVIIRERYRDNDSDLALG
MLAGAATGMALGSLFWVF*

SEQ ID. No: 46

FIGURE 24

>SCIDD116114045
CACCACAGAGGACCCCTTCAACCTAAGGAAACACCCAGGCTTCGATAGGACCATGCTGCAGAGGTGGCAGAAAAGGGAGA
TCAGCAACTTTGAATACCTCATGTACCTCAACACACTGGCCGGAAGGACCTACAATGACTACATGCAGTATCCCGTGTTT
CCCTGGGTCCTCGCTGACTACACCTCAGAGATGTTGAACTTGACGAATCCCAAGACTTTCCGGGATCTTTCTAAGCCAAT
GGGGGCTCAGACCAAGGAAAGGAAGTTGAAGTTTACCCAGAGGTTTAAAGATGTTGAAAAGATTGAAGGAGACATGACCG
TGCAGTGCCACTACTACACCCACTATTCCTCAGCCATCATTGTCGCTTCCTACTTGGTCCGAATGCCACCATTCACGCAG
GCCTTCTGCTCCTTACAGGGCGGAAGCTTTGATGTGGCTGATAGAATGTTCCACAGTGTAAAGAGCACGTGGGAGTCTGC
CTCCAAAGAGAACATGAGCGATGTCAGGGAGCTGACACCTGAATTCTTCTACCTGCCCGAGTTTTTAACCAACTGTAATG
CAGTGGAGTTTGGCTGCATGCAGGATGGAACGACACTGGGGGATGTGCAGCTTCCTCCCTGGGCTGACGGGGATCCGAGG
AAATTCATCAGCTTGCACAGACAGGCTCTGGAAAGTGACTTCGTCAGCAGCAACCTCCACCACTGGATAGACCTAATTTT
TGGGTATAAGCAGCAGGGGCCGGCTGCTGTAGAGGCAGTGAACACTTTCCACCCCTACTTCTACGGTGATAGAATAGACC
TGGGCAGCATCACTGACCCGCTGATCAAGAGCACCATCCTGGGCTTCATCAGCAACTTTGGACAGGTGCCCAAGCAGATC
TTCACTAAACCCCACCCATCCAGAAACACCACAGGGAAAAACCCAGGGCCTGGAAAGGATGCTTCCACCCCTGTAGGCCT
CCCAGGCCACTCACAGTCCTTCCTCCACAGCCTGCCAGCACTGAGACCCTCTCAGGTCACAGTCAAAGATATGTACCTTT
TCTCTCTAGGGTCGGAATCCCCCAAAGGGGCCATCGGCCACATCGTCCCTACTGAGAAGTCAATCCTGGCAGTGGAGAAG
AACAAGCTGCTGATGCCCCCTCTCTGGAACAGGACCTTCAGCTGGGGCTTTGATGACTTCAGTTGCTGCCTGGGGAGCTA
CGGCTCTGACAAGATCCTGATGACCTTTGAGAACCTGGCTGCCTGGGGTCCCTGTCTGTGCGCTGTATGCCCTTCCCCCA
CGATGATCGTCACATCCGGGGCCAGCGCAGTGGTGTGCATCTGGGAGCTGAGCCTGGTCAAAGGTCGCCCGAGAGGTCTG
AAACTCCGACAGGCCTTGTATGGACACACTCAGGCGGTCACATGTCTGACAGCCTCTGTCACCTTCAGCCTCCTGGTGAG
CGGATCCCAGGATCGCACTTGTATCCTGTGGGACCTGGACCACCTCTCTCGTGTGGCCTGCCTGCCTGTCCACCGGGAAG
GCATCTCAGCCATTGCCATCAGTGATGTCTCGGGAACCATTGTCTCCTGTGCCGGAGCCCACTTGTCCCTGTGGAATGTC
AATGGACAGCCCCTGGCCAGTATTACCACAGCCTGGGGCCCAGAAGGAACCATAACGTGCTGCTGCATAGTAGAGGGGCC
AGCGTGGGATGCAAGCCACGTGATCATCACGGGGAGTAAGGACGGAATGGTTCGGATTTGGAAGACAGAGGACGTGAAGA
TGCCTGTTCCCAGGCAGGCAGTGATGGAGGAGCCCTCCACGGAGCCCCTAAGCCCCAGAGGTCACAAGTGGGCCAAGAAT
CTTGCCCTGAGCCGAGAGCTGGATGTCAGTGTTGCTCTGAGTGGCAAGCCCAGCAAGGCAAGTCCTGCTGTGACAGCTCT
GGCCATCACTAGGAACCAGAGCAAGCTCTTGGTTGGCGATGAGAAGGGCGAATCTTCTGCTGGTCTGCTGATGGGTAGGA
GACAAGGAGCTGGAGGGACCGACCTGAAAGCCTGGAGCCCTGGGGTCGGCAGCAACAGGCTACAGGCACAAGATGATGTG
TAGCCTGGGCTGCTTAACCAGAGCAAGTTTTGGGGGGGCTCCACTCCACACAGTTCTCAAGGAGTCCCTGATGGTTTGCA
CCGTGTACCCTAAACATGTCTGTAGTCTATGGGACTTCTGTAAGAAGGATCTTGGTAGACACTGATGCTGGAAACTGACG
CTGCATGGGAAATTTCTACGTGGCTCACTTCACCAAGGCTTATTGCACTGGGAAAAGAAGAGGGGGTGGGTATTGGTTCA
TGGAAACCACCCCACTGTCTTTATTTTATTAAAACTCCATTTTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID. No: 47

FIGURE 24

>SCIDD116114045 Frame 2
TTEDPFNLRKHPGFDRTMLQRWQKREISNFEYLMYLNTLAGRTYNDYMQYPVFPWVLADY
TSEMLNLTNPKTFRDLSKPMGAQTKERKLKFTQRFKDVEKIEGDMTVQCHYYTHYSSAII
VASYLVRMPPFTQAFCSLQGGSFDVADRMFHSVKSTWESASKENMSDVRELTPEFFYLPE
FLTNCNAVEFGCMQDGTTLGDVQLPPWADGDPRKFISLHRQALESDFVSSNLHHWIDLIF
GYKQQGPAAVEAVNTFHPYFYGDRIDLGSITDPLIKSTILGFISNFGQVPKQIFTKPHPS
RNTTGKNPGPGKDASTPVGLPGHSQSFLHSLPALRPSQVTVKDMYLFSLGSESPKGAIGH
IVPTEKSILAVEKNKLLMPPLWNRTFSWGFDDFSCCLGSYGSDKILMTFENLAAWGPCLC
AVCPSPTMIVTSGASAVVCIWELSLVKGRPRGLKLRQALYGHTQAVTCLTASVTFSLLVS
GSQDRTCILWDLDHLSRVACLPVHREGISAIAISDVSGTIVSCAGAHLSLWNVNGQPLAS
ITTAWGPEGTITCCCIVEGPAWDASHVIITGSKDGMVRIWKTEDVKMPVPRQAVMEEPST
EPLSPRGHKWAKNLALSRELDVSVALSGKPSKASPAVTALAITRNQSKLLVGDEKGESSA
GLLMGRRQGAGGTDLKAWSPGVGSNRLQAQDDV*

SEQ ID. No: 48

FIGURE 24

>SC|DD64|14046
CAGAGGCAGGCAGGACAGGCACTGCASGCACTGAGAGCCTTGCAGTCAGAAGGGAAGCACASACAGGAAGAGGCAGTGAG
GAGAAAAGGAGCASAACTGAGACAAGCAATGGAGATCCCTGAGGAGTGCTGGCCAACGGCTGAAGTGTCCCTAAAAGACA
TCACTGAAATAATGGAGAGACATCTCAGTCACATGGAACGGACCCTGTCTCACAGTCAAAAGCTCTCAGATGGAGACCTG
GTAAGATGGGCATCTGGAGGGCTGGTCCTGCAGGGAATTTATAAGACCAACCACCCAAGAAGCCTGATTCAGAAGAGAGA
AGAGCTACTCAGTGTCCCTAAGCAGTTCTCACTTGCTGGCCCAGTACATGGCACAGAGATAAAAACAAAGGAATTTTCAT
CTTTTCAAGAACAAGCCATGTTTACACAGACTATAGAGAGGGTGGGTTTCAGTTCAACCCCCTTAGTTAAAGGAGAAGGT
TGGGGACTTAGTCTAGAAGCTGGTATGGGTCACAACAAACAGACAGAATCTGAAGATAACTACCAGTCCCATTCAAAGCA
AACTTATTTTTGCTCAGCCAGGTTCAGCTACATCCCATTGGCCACCTGCCATTTTCACATCAATGATCTTGAACTCTCCC
AGGCTGCTCTCCAGGAACTAAAAAGTATTGAAGAAATCCTGGAGCAGACTACACACCACCGAGATGGACTACCCTTACTG
AGGCACAGGGCTAAAAACTTTTTCCACAGGTTTGGCTCTCATGCTAACCAAGGCCCTGTGCACCTGGGGGGAATCTACTG
CTGGAAAGCCATTTCAGAAGGTTTCAAAAGTGAGCACTTGGCTGATGTAAAGCAGCAAGCAGAAGAGTCTTTGAATATTT
ACATTATGGGCAGTTATAGTGGCTTTGGAGTTAAAGTTGGTGCGAGTGTAAATATAACAAATTCAAAATCAAAAACAGCA
TTTTACAGTAAAACTCATCTAAACTCGCAAACCAAGGTACAACTATCTGTAGCCAAGATAGGTGGACCAGCAGAAGCAGA
TGGAATTGCCCAGTGGACAGCTGGCCTTGTAGCTAGCAATCAAACCTGGTCTGTTATTGATAGGAAACTGCAGTTGGTAC
CTATTTGGGACATTATCCTGTCCAGTCACAGAACTGAATTTAAGAATGCTCTTCAACTGGCTAACTGCCTCAAAGACCAC
TACACTGCTCTGACTGAACTAGCTGCCCAGATTCAAGAAGGGGAAGAATTTCTGACTGCTAGAAAAGAAGCTAAGCTTTT
CCTAAAGAATGTGAAAGGCTGGGAGGTTTCTGATCCTGAAGAACAGCTTAGGAAGTTAGTAGATTTTATGCAAACATTGA
GTCAAAAAATAAAAAGTTATGACATTTGGATTAACACATGCCTCATAGATTGGGATCTGCAAAATTTTCTAATAAACATT
GTCAACTTCTGCAAAAATTCACCCACTTATAAAACTCACTTTATTAAATCTCAGTTGTGCAGCCTTCTAGAACCTCATGT
CTACAAAGTGACAAACTTTCCTCAGGCACAATGCATCATACAGTGGATCAATCAGTCAGAGTCAGAGGAAGAACTAGTCA
AAATTACCTCATTTTCTGAATTCATTAACACCTTAAAGAAAACCCACAAATACCTAATGGAAGAGAGTTTCAAAACTGAG
CCCCCAGAAAGAGTGGAAGAAGCAAAGAGAATGGCTACATATGAAGTCACCACAGCTCTCAGCTCCTTCTTGAAGTACCT
CAGAGAAACACAGCAGCCAGACATGCAGCTGTTGCTACTCTCCATTGCTACTGGTGTAGGCTATCAGTTGGTAAACAGTA
TTTTTCAGCATCTTCTGGGGTGTGATGAGTTAAACTTCCTCTTGGATCAAATGGAAAATAACGAACATAAATACCAAGAA
CTGAAAAATATTTGCAATTACAGAGCCCAGGCATTCTTGGTGCTCACAGCCCTAAGAGCCACAGTTGAAATCACAGATGT
TTCTACAGAAGAGAAAGGACAACGTTTGACATTAATACAACAACATATGGGGTCACTGTTGTCTGAAGAAGTTGCACATG
TCCTCACAAAACATGGAGAACATCATGACTGGGAAAGGCTGGAGAATGATTTGAGATTACTCATTGAGGGGGACTATAAA
GCCACCACCCATTCCTTACAAATGGATGAAGTAAAAAAACAATTGCAAAGTTTNTGCCATGAAAAGAAACAGACTTATAA
ACAACAAGGTAATGAAAACAGAACAAAAGAAATGATAGAAAATGGACATTTCCTGGACTTACTCCAACGTTTAGGCCTAG
ACAATTACTATCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID. No: 49

FIGURE 24

>SC|DD64|14046 Frame 1
QRQAGQALXALRALQSEGKHXQEEAVRRKGAXLRQAMEIPEECWPTAEVSLKDITEIMER
HLSHMERTLSHSQKLSDGDLVRWASGGLVLQGIYKTNHPRSLIQKREELLSVPKQFSLAG
PVHGTEIKTKEFSSFQEQAMFTQTIERVGFSSTPLVKGEGWGLSLEAGMGHNKQTESEDN
YQSHSKQTYFCSARFSYIPLATCHFHINDLELSQAALQELKSIEEILEQTTHHRDGLPLL
RHRAKNFFHRFGSHANQGPVHLGGIYCWKAISEGFKSEHLADVKQQAEESLNIYIMGSYS
GFGVKVGASVNITNSKSKTAFYSKTHLNSQTKVQLSVAKIGGPAEADGIAQWTAGLVASN
QTWSVIDRKLQLVPIWDIILSSHRTEFKNALQLANCLKDHYTALTELAAQIQEGEEFLTA
RKEAKLFLKNVKGWEVSDPEEQLRKLVDFMQTLSQKIKSYDIWINTCLIDWDLQNFLINI
VNFCKNSPTYKTHFIKSQLCSLLEPHVYKVTNFPQAQSIIQWINQSESEEELVKITSFSE
FINTLKKTHKYLMEESFKTEPPERVEEAKRMATYEVTTALSSFLKYLRETQQPDMQLLLL
SIATGVGYQLVNSIFQHLLGCDELNFLLDQMENNEHKYQELKNICNYRAQAFLVLTALRA
TVEITDVSTEEKGQRLTLIQQHMGSLLSEEVAHVLTKHGEHHDWERLENDLRLLIEGDYK
ATTHSLQMDEVKKQLQSXCHEKKQTYKQQGNENRTKEMIENGHFLDLLQRLGLDNYYP

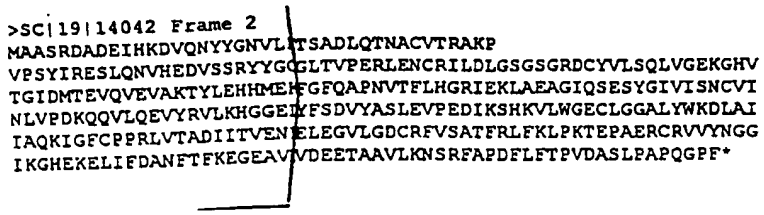
SEQ ID. No: 50

FIGURE 24

>SC|19|14042
TCTTCGAAAGCCGGGCTGAGGGGAATCCTGGACAGGGGAATCCTGGACGTGGAGATCGTGAGTCATGGCTGCTTCCCGAG
ACGCTGATGAGATCCACAAGGACGTTCAGAACTACTATGGGAATGTACTGAAGACATCTGCAGACCTCCAGACTAATGCT
TGTGTCACGCGAGCCAAGCCGGTCCCCAGCTACATCCGGGAAAGTCTGCAGAATGTACACGAAGACGTTAGTTCGAGGTA
TTATGGCTGTGGTCTGACTGTTCCTGAGCGGCTGGAAAACTGCCGAATTTTGGATCTGGGTAGTGGGAGTGGCAGGGATT
GCTATGTGCTTAGCCAGCTGGTTGGTGAGAAGGGACATGTCACCGGAATAGACATGACTGAGGTCCAGGTCGAAGTGGCT
AAAACCTATCTTGAACACCACATGGAAAAATTTGGTTTCCAGGCACCCAATGTGACTTTTCTCCACGGCCGCATCGAGAA
GTTGGCAGAGGCTGGGATCCAGAGTGAGAGCTATGGTATTGTCATATCCAACTGTGTTATCAACCTTGTTCCTGATAAAC
AACAAGTCCTCCAGGAGGTCTATCGAGTGCTGAAGCACGGCGGGGAGCTCTATTTCAGTGACGTCTATGCCAGCCTTGAA
GTGCCAGAAGACATCAAGTCGCACAAAGTTTTATGGGGGGAATGCCTGGGAGGCGCTCTGTACTGGAAGGATCTTGCCAT
CATTGCCCAAAAGATTGGGTTCTGCCCTCCACGTTTGGTCACTGCCGATATCATTACTGTTGAAAACAAGGAGCTCGAAG
GGGTTCTTGGTGACTGTCGCTTTGTGTCTGCCACATTTCGCCTCTTCAAACTCCCTAAGACAGAGCCAGCCGAAAGATGC
CGAGTTGTTTACAATGGAGGAATCAAGGGACATGAAAAGGAACTAATTTTCGATGCAAATTTCACATTCAAGGAAGGCGA
AGCTGTTGCAGTGGATGAGGAGACGGCAGCTGTCCTGAAGAACTCACGTTTTGCTCCGGATTTTCTCTTCACACCTGTTG
ACGCCTCGCTGCCAGCTCCCCAGGGGCCGTTCTGAGTTAGAGACAAAGGTTCTAATCAGAGATCCATTCAAGCTTGCAGA
GGACTCTGACAAGATGAAGCCCAGACATGCACCTGAAGGCACGGGAGGCTGCTGTGGCAAGAGGAAAAACTGCTAGATCT
ACAGCCAGCGCGGAGCCCACCGGGCTCAAGAGGGTGGCTAAAGGACAGTCACAGAGGCTTCTTAGCCTGCTCTTCGCCAG
TGCACAGATTATGTGAAGGTGGCAAAGCCACCACAAGCTAGACCACTGCTAAGAATAAGAGTGACTTTTAGAGGATGTTA
ATTGAAGGTTCACAGCAAATCGCCTGCTTTTCTATTTCTCTATCTCAGAGTTCTGGTGCCACCTAGTGGTCAGAAGTAGA
ACTTGGAAGCCCAAGGTTTACTCAAAGGGCCAAAGGCATCATCAACGTTGTGAGAATTATCTTCCTTCTGGCCTACCACA
GGACACCTCTGGGTTCTTCTCTGTGGTTACCAGGAAGCACAGTACTTACTAAATTTATGCTAACCATGACAAAAGATTGT
CAACTCAAATTTGCTAGGAGTATTCTTTAGGTTGCTGTCTGCAATTTTTTTCTCTGTAACTGAATGAAAAAGAAAACAAA
TAAAAAATAAATTTGACTTCGAAAAAAAAAAA

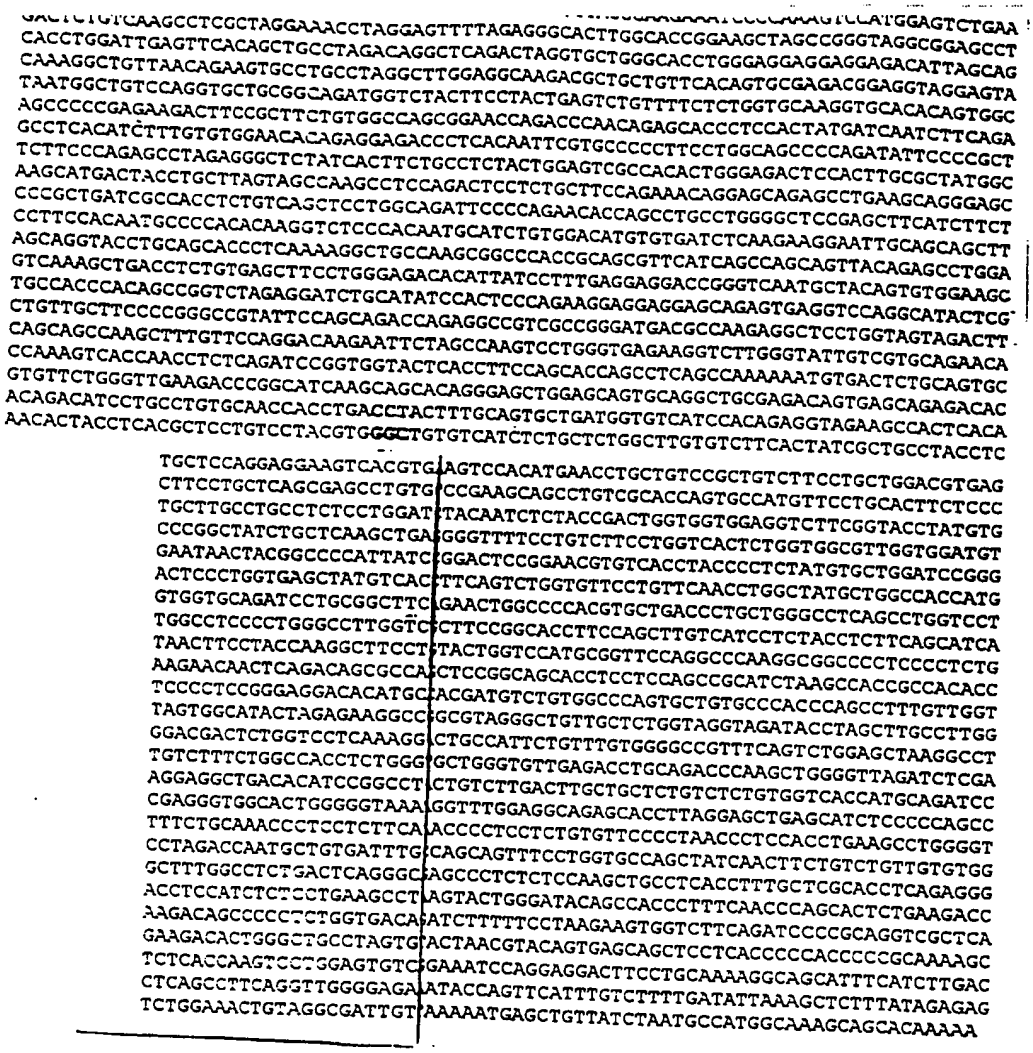
SEQ ID. No: 51

FIGURE 24



SEQ ID. No: 52

FIGURE 24



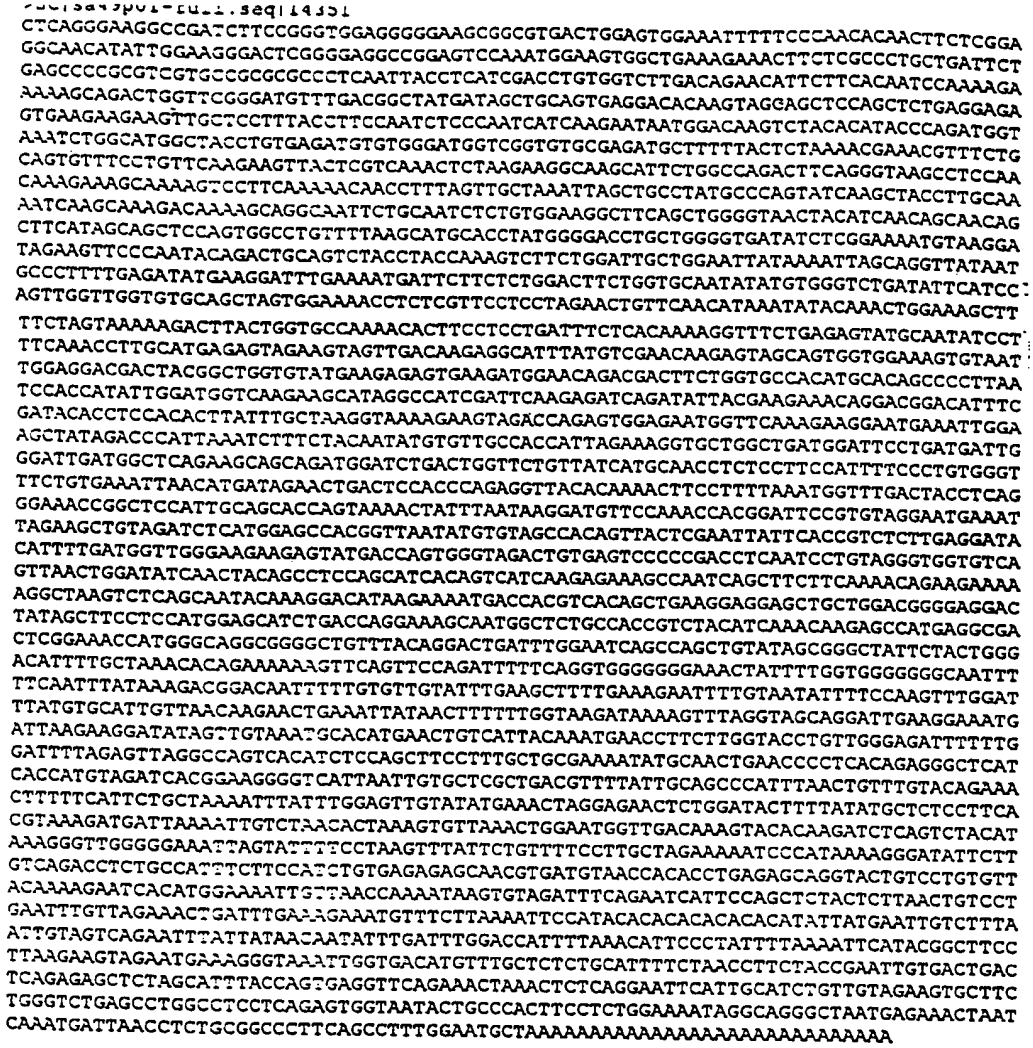
SEQ ID. No: 53

FIGURE 24

>SC|28LONG|14041 Frame 2
MAVQVLRQMVYFL
LSLFSLVQGAHSGSPREDFRFCGQRNQTQQSTLHYDQSSEPHIFVWNTEETLTIRAPFLA
APDIPRFFPEPRGLYHFCLYWSRHTGRLHLRYGKHDYLLSSQASRLLCFQKQEQSLKQGA
PLIATSVSSWQIPQNTSLPGAPSFIFSFHNAPHKVSHNASVDMCDLKKELQQLSRYLQHP
QKAAKRPTAAFISQQLQSLESKLTSVSFLGDTLSFEEDRVNATVWKLPPTAGLEDLHIHS
QKEEEQSEVQAYSLLLPRAVFQQTRGRRRDDAKRLLVVDFSSQALFQDKNSSQVLGEKVL
GIVVQNTKVTNLSDPVVLTFQHQPQPKNVTLQCVFWVEDPASSSTGSWSSAGCETVSRDT
QTSCLCNHLTYFAVLMVSSTEVEATHKHYLTLLSYVGCVISALACVFTIAAYLCSRRKSR
DYTIKVHMNLLSAVFLLDVSFLLSEPVALTGSEAACRTSAMFLHFSLLACLSWMGLEGYN
LYRLVVEVFGTYVPGYLLKLSIVGWGFPVFLVTLVALVDVNNYGPIILAVRRTPERVTYP
SMCWIRDSLVSYVTNLGLFSLVFLFNLAMLATMVVQILRLRPHSQNWPHVLTLLGLSLVL
GLPWALVFFSFASGTFQLVILYLFSIITSYQGFLIFLWYWSMRFQAQGGPSPLKNNSDSA
KLPISSGSTSSSRI*

SEQ ID. No: 54

FIGURE 24



SEQ ID. No: 55

FIGURE 24

SC|sa49p01-full.seq|14351 Frame 2
MFDGYDSCSEDTSSSSSSEESEEEVAPLPSNLPI
IKNNGQVYTYPDGKSGMATCEMCGMVGVRDAFYSKTKRFCSVSCSRSYSSNSKKASILAR
LQGKPPTKKAKVLQKQPLVAKLAAYAQYQATLQNQAKTKAGNSAISVEGFSWGNYINSNS
FIAAPVACFKHAPMGTCWGDISENVRIEVPNTDCSLPTKVFWIAGIIKLAGYNALLRYEG
FENDSSLDFWCNICGSDIHPVGWCAASGKPLVPPRTVQHKYTNWKAFLVKRLTGAKTLPP
DFSQKVSESMQYPFKPCMRVEVVDKRHLCRTRVAVVESVIGGRLRLVYEESEDGTDDFWC
HMHSPLIHHIGWSRSIGHRFKRSDITKKQDGHFDTPPHLFAKVKEVDQSGEWFKEGMKLE
AIDPLNLSTICVATIRKVLADGFLMIGIDGSEAADGSDWFCYHATSPSIFPVGFCEINMI
ELTPPRGYTKLPFKWFDYLRETGSIAAPVKLFNKDVPNHGFRVGMKLEAVDLMEPRLICV
ATVTRIIHRLLRIHFDGWEEEYDQWVDCESPDLNPVGWCQLTGYQLQPPASQSSRESQSA
SSKQKKKAKSQQYKGHKKMTTSQLKEELLDGEDYSFLHGASDQESNGSATVYIKQEP*

SEQ ID. No: 56

FIGURE 24

>SCIsa61|14347
GGGGCTTCACTCTCCAGCCTTGGAGAGCAGTCAGGGAAGGCCTCCAGGAGGAAGCAGCTACCTGTGGCAGGGCAGAGCAG
TGCCTGTACCAGTCTCCCGGAACTTCAGAACTGCGGTGGCCATGGAGTCTGTGGAGCCCCCGGTCAAAGACGGCATCCTC
TACCAGCAGCACGTAAAGTTTGGCAAGAAATGCTGGCGCAAAGTGTGGGCTCTGCTGTATGCGGGAGGCCCATCAGGGGT
AGCTCGGCTAGAAAGCTGGGACGTGCGTGATGGTGGCCTGGGACCAGCAGGCGACAGGTCCACAGGGCCCAGCCGTCGAG
GGGAACGCCGGGTCATACGCTTGGCTGACTGTGTATCTGTCCTGCCTGCGGATGGCGAGAGCTGTCCCAGGGACACTGGT
GCCTTCCTGATTACCACCACTGAGCGAAGCCACCTGTTGGCTGCACAGCACCGCCAGTCCTGGGTGGACCCCATCTGTCA
GCTGGCCTTCCCGGGTACCGGAGAATGTTCGTCAGGATCAGGACAGGCTGAGAATCCAAAAAGGGGCTTTGTTCCCATGG
AAGAAAACTCTATCTACTCCTCCTGGCAGGAAGTGACCGAATTTCCGGTGATCGTGCAGAAGACAGAAGCCACCTCCCGC
TGCCAGCTGAAAGGACCCTACCTCCTGGTGCTGGGCCAAGATGACATCCAACTGAGGGAGACATCCAAGCCCCAGGCCTG
TTTTAGCTGGCCCTACCGTTTCCTGCGCAAGTACGGCTCTGACAAGGGTGTGTTCTCGTTTGAGGCTGGCCGCCGCTGTG
ACTCAGGTGAGGGCCTTTTTGCCTTCAGTAGCCCGCGTGCCCCAGACATATGTGGGGTTGTGGCTGCCGCCATTGCCCGC
CAGCGGGAGCGTCTTCCAGAGCTGGCCATGTCCCCACCCTGCCCCCTGCCTCGGGCCCTCTCCCTGCCCTCCCTAGAGCC
CCCTGGAGAGCTTCGGGAGGTGGCCCCAGGATTTGAGCTGCCCACTCCCAGAAAGCTGCCTCTAACTGATCCCGGGCCTC
AAAGCCTACCATTGCTGCTCAGCCCCAACACAAGAAGGACCGGCATCCGGTCTCTATGCGTCCGTGTGCAAGCAGACCAG
CAAGCACACAGGCACGGCGGAGCATTTCTATGAGAACGTGTGCATGCTGGAGGCCAGCCTTGGGCTGACCAATGGGGGTC
CTGAAGCCCAAGAGGGCCCCCTTGGTGGCCGCAGCCCCCTTGGGCAGCCTTATCTACCATAACACTGAGGATCTGAGTTG
GCCGGGCTCGGCCCAGGACAGCAATCTGGAAGCCCAGTACCGGAGGCTGCTGGAACTGGAGCTGGATGAGGCCGGAAGCG
CCGGCCGCTCTGGAGCGCAGGCAGGCATCAAGGCCAAGCTGGTGACCCTGCTGACCCGTGAACGGAAGAAGGGCCCCGCC
CCCTGTGACCGGCCCTGAAGGCCTGAGCGGCCAGCCACTGCAGGACAGAGGTGATCACCCAAGACCAGGAACAACTTCGA
ACATAACCCGTCTACTCTGACCTGCAGGGACAAGCCAGGTGGCCCGGGGAGGAGCCACACTCTGCCCTACCTCCTCCCTC
AGACTGTACAGATTGAACAGTAATAAAGCTTGCCTATCAACTTCAAAAAA

SEQ ID. No: 57

FIGURE 24

SC|sa61|14347 Frame 2
MESVEPPVKDGILYQQHVKF
GKKCWRKVWALLYAGGPSGVARLSWDVRDGGLGPAGDRSTGPSRRGERRVIRLADCVSV
LPADGESCPRDTGAFLITTTERSHLLAAQHRQSWVDPICQLAFPGTGECSSGSGQAENPK
RGFVPMEENSIYSSWQEVTEFPVIYQKTEATSRCQLKGPYLLVLGQDDIQLRETSKPQAC
FSWPYRFLRKYGSDKGVFSFEAGRCDSGEGLFAFSSPRAPDICGVVAAAIARQRERLPE
LAMSPPCPLPRALSLPSLEPPGELEVAPGFELPTPRKLPLTDPGPQSLPLLLSPNTRRT
GIRSLCVRVQADQQAHRHGGAFLYRVHAGGQPWADQWGS*

SEQ ID. No: 58

FIGURE 24

>SC|smc34|14348
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CCAACAGGACATCTTACTATCGAAACCCGCTCTGTGAGCCAGGATCATCAGGGGCCTCTGGTGGAGGCCACTCTTCTAGT
GCATCCGTGAGCAGCGTCCGTTCTCGAAGCAGGACCACTTCTGGGACGGGCCTCTCCAGCCCTCCGCTGGCCGCGCAGAC
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GGCTTCCATGGGACCCCGAGAGGCAGTCAGTGGCCTGGCAAGGAGTCGGGACTACTTTCTGACACTGCGGGAGACTTGGA
AGCAGCATACACGACAGCTATATGGCCCGGAAGCCATGCCCACCCATGCCTGCTGCTTGTCACCCAGCCTCATTCGCAAT
GAAGTTGAGTTCCTCAAAATGGACTTCAATTGGCGAATGAAGGAAGTACTTGTCAGCTCCATGCTGAGTGCCTACTATGT
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CTGGCGTGCTGGCAGAAGGTGGACCCAGCTCTGTGCTCCAATGTGCTGCAGCCACCGTGGACTGAAGAGTGCATGTGGCC
ACAGGGTGTGCTGGTGAAACACAGCAAGAATGTCTACAAAGCAGTGGGCCACTACAACGTGGCATCCCCTCCGATGTCTC
CCACTTCCGCTTCCATTTCTTTTTCAGCAACCCCCTGCGGATCCTCAACATCCTTTTGCTGCTGGAGGGTGCCGTCATTG
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TATGCCTTCTTCAAGCTGCTCCGGGACCGCCTGGTATTGGGCAAAGCTACTCCTACTCAGCCAGCCCCCAGAGGACCTGG
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TGTTACGTACTGTTTCTTTCTTTGATAATTGATGTGGTAAGAAAAAAGTCTTATTTTTATACTCCAAAAA

SEQ ID. No: 59

FIGURE 24

SC|smc34|14348 Frame 2
MGGRRGPNRTSYYRNPLCEPGSSGASGGGHSSSASVSSVR
SRSRTTSGTGLSSPPLAAQTVVPLQHCKIPELPVQASILFELQLFFCQLIALFVHYINIY
KTVWWYPPSHPPSHTSLNFHLIDFNLLMVTAIVLGRRFIGSIVKEASQRGKGLPLPLHPA

VPHPLHVLTATGWSLCRSLIHLFRTYSFLNLLFLCYPFGMYIPFLQLNYDLRKTNLFTHM
ASMGPREAVSGLARSRDYFLTLRETWKQHTRQLYGPEAMPTHACCLSPSLIRNEVEFLKM
DFNWRMKEVLVSSMLSAYYVAFVPVWFVKNTHYYDKRWSCELFLLVSISTSVILMQHLLP
ASYCDLLHKAAAHLACWQKVDPALCSNVLQPPWTEECMWPQGVLVKHSKNVYKAVGHYNV
ASPPMSPTSASISFSATPCGSSTSFCCWRVPSLSTSCTP*

SEQ ID. No: 60

FIGURE 24

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CCTTGGCCAGCCCTGGGCAGGGGCTGCTGCTGATTCACAGGCTGTGGTGTGCGAGGGGACTGCCTGCTATACAGCCCATT
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CTGTCCTTGACACCTCACCCCAGCCATCTGCCCAAGTGGCATGAGAGTCCCTGTGGGACCCCCGAAGCTCCAGGTAACAG
CATTGAAGGTTTCCTGTGCAAGTTCAACTTCAAAGGCATGTGTAGGCCACTGGCGCTGGGTGGTCCAGGGCGGGTGACCT
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GAAGCTAAGAGTGAAACCCACTATTTCCTATGCAATGAAAAGACTCCAGGAATATTTCACTGGGGCAGCTCAGGCCCACT
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GGAGCTTCCACTGTGAATGTTGGGTTGGTTACCAACCCAGTGGCCCCAAGGAAGAGGCCTGTGAAGATGTGGATGAGTGT
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AACGGAGAGCCAAGAAGGAGGAGATAAAAGAGAAGAAGCCTCAGAATGCAGCCGACAGCTATTCCTGGGTTCCAGAGCGA
GCAGAGAGCCAAGCCCCGGAGAATCAGTACAGCCCAACACCAGGGACAGACTGCTGAAGACTATGTGGCCTTAGAGACAG
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CCAATAGCAACTGGTTCCTCTGGTTCCTAATTAGGGGGAAAGGAGTCAGAGGGGTGGGACAGGGTGGGGGGATGGGGCTT
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TAAGCACCTCTCCTAATAAACTCTGAAAAAA

SEQ ID. No: 61

FIGURE 24

SC|AA4.1|15737 Frame 3
MAISTGLFLLLGLLGQPWAG
AAADSQAVVCEGTACYTAHWGKLSAAEAQHRCNENGGNLATVKSEEEARHVQQALTQLLK
TKAPLEAKMGKFWIGLQREKGNCTYHDLPMRGFSWVGGGEDTAYSNWYKASKSSCIFKRC
VSLILDLSLTPHPSHLPKWHESPCGTPEAPGNSIEGFLCKFNFKGMCRPLALGGPGRVTY
TTPFQATTSSLEAVPFASVANVACGDEAKSETHYFLCNEKTPGIFHWGSSGPLCVSPKFG
CSFNNGGCQQDCFEGGDGSFRCGCRPGFRLLDDLVTCASRNPCSSNPCTGGGMCHSVPLS

ENYTCRCPSGYQLDSSQVHCVDIDECQDSPCAQDCVNTLGSFHCECWVGYQPSGPKEEAC
EDVDECAAANSPCAQGCINTDGSFYCSCKEGYIVSGEDSTQCEDIDECSDARGNPCDSLC
FNTDGSFRCGCPPGWELAPNGVFCSRGTVFSELPARPPQKEDNDDRKESTMPPTEMPSSP
SGSKDVSNRAQTTGLFVQSDIPTASVPLEIEIPSEVSDVWFELGTYLPTTSGHSKPTHED
SVSAHSDTDGQNLLLFYILGTVVAISLLLVLALGILIYHKRRAKKEEIKEKKPQNAADSY
SWVPERAESQAPENQYSPTPGTDC*

SEQ ID. No: 62

FIGURE 24

SC|HDD2|16960
CCCATTACTTCCACCTCCCATGAGGACTGCACCTGCGGCAGCGATTTTATGTGAACTTGGATTACTGTATGGAAATGGAG
GAGTGAGAAAGTGTGGGAATCATAAAAGAAGGCAGAACCAAAAGACAGAGGGATCTTGGGCCATGAGTCACAGCAAGGAA
AGCCTCCCACCAAACATCTGCACTGGACTCCTACAGGAACAAGAAATGAACTATCGTGTTCAGCCCCTGAGACTTGGGCT
TAACATGATTTTCACATTGTCTACCCTACCTAATAGAGCAGAGATGTAAATATTATTCTTATTTTAGAGGTGTGATGCCT
CAGCTGCAATGGGTGAGAACTACTCCTCATTTATTCTCTTCCAAGGCAATAAAAGAGAATGGACCAAAGACAGTCTGTCA
TCACATCTAGTCAAAAGAGCTAATGTCGCAGTACAACTCTTCAAAAGAAAAAGAAAAAACAAGAAAAAAGTAATAAACAG
ATGTGTTCTGCTTGAAAAA

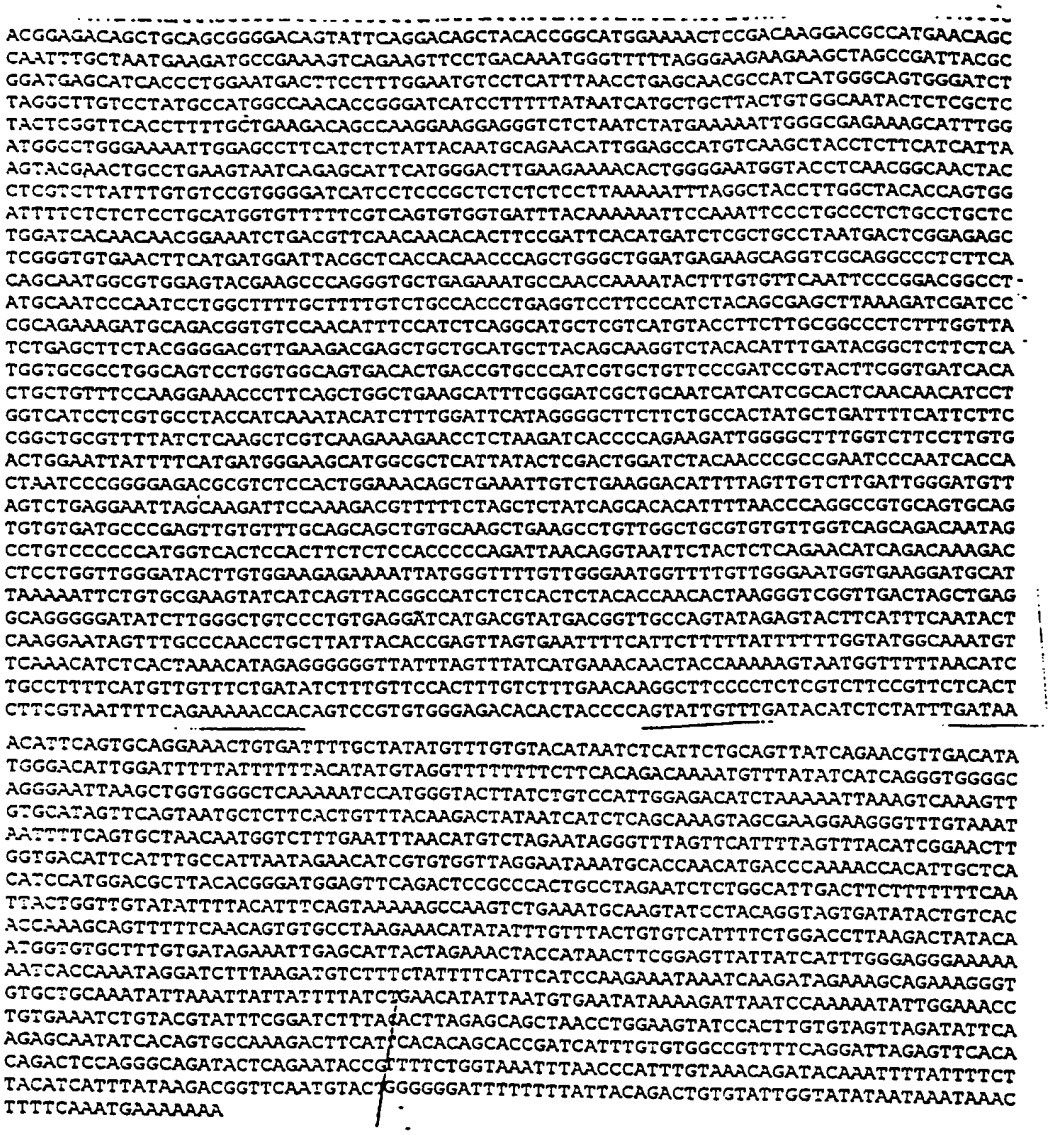
SEQ ID. No: 63

FIGURE 24

>SC|HDD2|16960 Frame 2
MRTAPAAAILCELGLLYGNGGVRKCGNHKRRQNQKTEGSWAMSHSKESLPPNIC
TGLLQEQEMNYRVQPLRLGLNMIFTLSTLPNRAEM*

SEQ ID. No: 64

FIGURE 24



SEQ ID. No: 65

FIGURE 24

SC|aftp1|15065 Frame 2
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ESQKFLTNGFLGKKKLADYADEHHPGTTSFGMSSFNLSNAIMGSGILGLSYAMANTGIIL
FIIMLLTVAILSLYSVHLLLKTAKEGGSLIYEKLGEKAFGWPGKIGAFISITMQNIGAMS
SYLFIIKYELPEVIRAFMGLEENTGEWYLNGNYLVLFVSVGIILPLSLLKNLGYLGYTSG
FSLSCMVFFVSVVIYKKFQIPCPLPALDHNNGNLTFNNTLPIHMISLPNDSESSGVNFMM
DYAHHNPAGLDEKQVAGPLHSNGVEYEAQGAEKCQPKYFVFNSRTAYAIPILAFAFVCHP
EVLPIYSELKDRSRRKMQTVSNISISGMLVMYLLAALFGYLSFYGDVEDELLHAYSKVYT
FDTALLMVRLAVLVAVTLTVPIVLFPIRTSVITLLFPRKPFSWLKHFGIAAIIIALNNIL
VILVPTIKYIFGFIGASSATMLIFILPAAFYLKLVKKEPLRSPQKIGALVFLVTGIIFMM
GSMALIILDWIYNPPNPNHH*

SEQ ID. No: 66

FIGURE 24

>SCIF77112685
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TCAACGCCGACGAGCTACGGGGTCGCTTCCAGGACCTGCTGAGCCGGCTGCATGCCAACCAGAGCCGAGAGGACTCGAAC
TCAGAACCAAGTCCTGACCCAGCTGTCCGGATACTCAGTCCAGAGGTGAGATTGGGGTCCCACGGCCAGCTGCTACTCCG
CGTCAACCGGGCGTCGCTGAGTCAGGGTCTCCCCGAAGCCTACCGCGTGCACCGAGCGCTGCTCCTGCTGACGCCGACGG
CCCGCCCCTGGGACATCACTAGGCCCCTGAAGCGTGCGCTCAGCCTCCGGGGACCCCGTGCTCCCGCATTACGCCTGCGC
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CTGCTAATAAAGGTGAGCTTGGTTTCAAAA

SEQ ID. No: 67

FIGURE 24

>SC|F77|12685 Frame 1
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GRFQDLLSRLHANQSREDSNSEPSPDPAVRILSPEVRLGSHGQLLLRVNRASLSQGLPEA
YRVHRALLLLTPTARPWDITRPLKRALSLRGPRAPALRLRLTPPPDLAMLPSGGTQLELR
LRVAAGRGRRSAHAHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMCVGE
CPHLYRSANTHAQIKARLHGLQPDKVPAPCCVPSSYTPVVLMHRTDSGVSLQTYDDLVAR

SEQ ID. No: 68

FIGURE 24

SEQUENCE LISTING

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Moore, Kateri

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<211> 743
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<213> Mus musculus

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<210> 8
<211> 174
<212> DNA
<213> Mus musculus

<400> 8
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<210> 9
<211> 711
<212> DNA
<213> Mus musculus

<400> 9
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<210> 10
<211> 356
<212> DNA
<213> Mus musculus

<400> 10
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gcacatcata ncnnttgatg agctgaagac cgactacaag aaaccctata gaccagtgca 240
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<210> 11
<211> 400
<212> DNA
<213> Mus musculus

<400> 11
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tgctgctang ggactggc aggctcgttt caaaccggcc 400

<210> 12
<211> 400
<212> DNA
<213> Mus musculus

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<211> 1360
<212> DNA
<213> Mus musculus

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<211> 1712
<212> DNA
<213> Mus musculus

<400> 14
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<211> 1539
<212> DNA
<213> Mus musculus

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<210> 16
<211> 3599
<212> DNA
<213> Mus abbotti

<400> 16
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<210> 17
<211> 399
<212> DNA
<213> Mus musculus

<400> 17
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<210> 18
<211> 400
<212> DNA
<213> Mus musculus

<400> 18
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<210> 19
<211> 2313
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<213> Mus musculus

<400> 19
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caacaaccag attcttgaag tgaaaagtcc nataaagcaa agcaaatcag ataagcaaat 300
aaagaatggt gaatgtgaca aggcatacct agatgaactg gtngagcttc ccagaangtt 360
natgacattg agagaaagac acattctgca gcagatcgtg aaccttatng aagaaactgg 420
gacactttca tatcaccaac acaacatttg attttgatct ttgctcgctg gacaaaacca 480
cagtccgtta actacagagt tcctggaaac atctggaaca tcctgaagat ataaccactg 540
gatgcctcna gaactaatgt gtttnnnnnn nnnnngggtt nnnnnntttg ggtgtgaatt 600
tttgttcccg ttgtttaaat gaaaaccccc cnaatgatgc n                     641

<210> 31
<211> 400
<212> DNA
<213> Mus musculus

<400> 31
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gcaggggaga cccagcggca gattctgtca cagacgaggg agaaggcgtg aggagacaaa 180
gccgtcacat ccgcgacagc ttccttcagc agcctcctcc tctccagtcc agagccgacc 240
cccgagcccc tgaggcatcc cttccgtctt cggaacaccc tagtcattca ttgctaacag 300
gggatcatga gggaccctgt aagtagccag tacagctcct ttcttttctg gangatgccc 360
atcccacaac tggatctgtc ngagctggaa ggcctgggcc                       400

<210> 32
<211> 400
<212> DNA
<213> Mus musculus

<400> 32
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tggtgcaagc tgtgcangcg gttcatcttg antctgacgc tttcctantt tgtctcaacc 180
atgctctgag cacagaaaan gangaagtga tgggtctgtg tataggggan ttgaatgatg 240
acataaggag tgactccnaa tttacataca ctggaacgga aatgcgcaca gtccnagaaa 300
agatggatac catcagaatt gttcatatcc attctgtcat catcttgcgg cgttctgaca 360
aganaaanga ccgtgtnnaa atttctccan ancanctgtc                       400

<210> 33
<211> 400
<212> DNA
<213> Mus musculus

<400> 33
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catgacactt acaaggtgga caggcatgat tattgggcca ccaaggacaa actatgaaaa 180
cagaatatat agcctgaaag tagaatgtgg atctaaatac ccagaagctc ctccatcagt 240
tagatttgta acaaaaatta atatgaatgg gatcaataat tccagtggaa tggtggatgc 300
acggagcata ccagtattag caaaatggca aaattcctat agcattaaag tcatacttca 360
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<210> 34
<211> 400
<212> DNA
<213> Mus musculus

<400> 34
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cgcgcaccaa aaagggaatt ctcctgtttg ctganattat actgtgcctg gtgatcttga 180
tttgcttcag tgcatctaca acatcggcct actcctccct gtcggtgatt gagatgatct 240
gtgctgctgt cttacttgtc ttctacacgt gtgacctgca ctccaagata tcattcatca 300
actggccttg gactgacttc ttcagatccc tcataacaac catcctgtac ctgatcacct 360
ccattgttgt ccttgtagaa agaagaagca gctccagagt 400

<210> 35
<211> 400
<212> DNA
<213> Mus musculus

<400> 35
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gaaaaaggga ggagatggaa ttaaaccgcc tccaattatt ggaagatttg gaacctcact 180
gaaaattggt atcgttggat tgccaaatgt tgggaaatct accttcttca atgtattaac 240
caatagtcag gcttcancag aaaacttccc attctgcact attgatccta atgagagcag 300
agtgcctgtg ccagatgaga ggttcgactt tcttttgccag taccataaac cancaagcaa 360
gatcctgctt tcctaaatgt aatggatatt gctggccttg 400

<210> 36
<211> 400
<212> DNA
<213> Mus musculus

<400> 36
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tgtnaatccg aangcctatc ccctcncana tnccccacctc accaagaanc tgctggacct 180
tgttcnacag tcttgttnct acaancagct tcngaaagga ccnatgaacc nccaaaaccc 240
tcaacagaag catctctgan ttcattgtga tgcngcanan ctgaaccttg gagatcccct 300
gcacctcccct ctnctgtcga aaaaaanaat gtcccctacct nttttgtncnn ccaacaggct 360
ttangaaagg cctntgggtt tccagccntc atcccctgtct 400

<210> 37
<211> 394
<212> DNA
<213> Mus musculus

<400> 37
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ccaggcttcc tcngccccca naaagccacc anacctcaca tggaaccaaa ggcccatgc 180
ccanccgccg tcccctcaga gganangaaa tttcgtgttc ttgtnggcgt cactggcagc 240
gtggccgctc tgaagctgcc tctcctgnta tctannctgt tggacgttcc tgngatgtgg 300
aagcgccgtt ctgacccggt tctccacatt gacctgcgga tgtgggctga cctcatgcta 360
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<210> 38
<211> 315
<212> DNA
<213> Mus musculus

<400> 38

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nangcagatt cgattccggt ttgatggaca accaatcaat gaaacagaca ctccacccca 180
nctggagatg gaggatgagg acaccnttga tgtattccag cagcagacag gaggatcanc 240
ctcccgaggg agcgtcccac acccaaccgt tgtcctgacc tgtgctattg agcagtgacc 300
atgctnccac accca 315

<210> 39
<211> 400
<212> DNA
<213> Mus musculus

<400> 39
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ttcagatccc caggggagcg gcctctacag caaccttcag cagtatgaca tcccgtaccc 240
tgaagccatc tttgaacttg gctttttctt tcacaacccc aagccctttt tcatgttggc 300
caaggactgt ncctgngca ctacaggcca atgtcactca ctacttcctg aagctcctcc 360
acgacangga ccgcttctgc gctctntaca canaaatcga 400

<210> 40
<211> 349
<212> DNA
<213> Mus musculus

<400> 40
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caacactaag gcagccagtg ctgaagggaa agagggctgt gagtcgctct cgtgtttacc 120
tcccgtgtca ctgcttccac acganaaaga caagccggtt gctgaaccaa tccccatctg 180
tagcttctgt cttggtacaa aagaacagaa ccgggagaag cagcccgagg aactcgtctc 240
ctgcgcggac tgcggcaata ncggtcatcc atcgtgttta aagttctccc canagctnac 300
agtgagagtg aagccttacn gtggcttnca ttgantgtaa aacatcact 349

<210> 41
<211> 397
<212> DNA
<213> Mus musculus

<400> 41
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gccggagccg cccaggccac cccctctcat gaagccaaag gcagcagtgg cagcagcacg 120
gtacagcggt ctaagtcctt tagcttgcgg gctcaggtga aggagacctg tgcagcctgc 180
cagaagactg tgtacccgat ggagcggctg gtggcagaca agctcatttt ccacaactct 240
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ggtgaatttt actgcagacc tcactttcag cagctgttta agagtaaagg caactacgat 360
gaaggttttc gtaaacagca caaggactct gggccac 397

<210> 42
<211> 158
<212> PRT
<213> Mus musculus

<400> 42
Met Phe Gln Ala Ala Gly Ala Ala Gln Ala Thr Pro Ser His Glu Ala
1 5 10 15

Lys Gly Ser Ser Gly Ser Ser Thr Val Gln Arg Ser Lys Ser Phe Ser
20 25 30

Leu Arg Ala Gln Val Lys Glu Thr Cys Ala Ala Cys Gln Lys Thr Val
35 40 45

Tyr Pro Met Glu Arg Leu Val Ala Asp Lys Leu Ile Phe His Asn Ser

```
     50                  55                  60

Cys Phe Cys Cys Lys His Cys His Thr Lys Leu Ser Leu Gly Ser Tyr
 65                  70                  75                  80

Ala Ala Met His Gly Glu Phe Tyr Cys Arg Pro His Phe Gln Gln Leu
                85                  90                  95

Phe Lys Ser Lys Gly Asn Tyr Asp Glu Gly Phe Gly Arg Lys Gln His
            100                 105                 110

Lys Glu Leu Trp Ala His Lys Glu Val Asp Ser Gly Thr Lys Thr Ala
        115                 120                 125

Asp Pro Phe Asn Thr His Ser Leu Pro Ala His Gly Leu Pro Leu Gly
    130                 135                 140

Ser Gly Lys Glu Ile Asn Pro Gly Ala Arg Gly Gly Arg Gly
145                 150                 155
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<210> 43
<211> 1360
<212> DNA
<213> Mus musculus

<400> 43

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acagaatctt aaacccactg aatggatggg aacagctggc agagctttct aggacctcca 180
cctctggtgg caacccctcc tttgtgcctc tctccaagtt catgaacacc cagtattttg 240
gaactattgg tttgggaacg cctcctcaga atttcaccgt tgtctttgac acgggttctt 300
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<210> 44
<211> 419
<212> PRT
<213> Mus musculus

<400> 44

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Glu Pro Glu Glu Ala Lys Leu Ile Arg Val Pro Leu Gln Arg Ile His
            20                  25                  30

Leu Gly His Arg Ile Leu Asn Pro Leu Asn Gly Trp Glu Gln Leu Ala
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         35                  40                  45

Glu Leu Ser Arg Thr Ser Thr Ser Gly Gly Asn Pro Ser Phe Val Pro
     50                  55                  60

Leu Ser Lys Phe Met Asn Thr Gln Tyr Phe Gly Thr Ile Gly Leu Gly
 65                  70                  75                  80

Thr Pro Pro Gln Asn Phe Thr Val Val Phe Asp Thr Gly Ser Ser Asn
                 85                  90                  95

Leu Trp Val Pro Ser Thr Arg Cys His Phe Phe Ser Leu Ala Cys Trp
            100                 105                 110

Phe His His Arg Phe Asn Pro Lys Ala Ser Ser Ser Phe Arg Pro Asn
        115                 120                 125

Gly Thr Lys Phe Ala Ile Gln Tyr Gly Thr Gly Arg Leu Ser Gly Ile
    130                 135                 140

Leu Ser Gln Asp Asn Leu Thr Ile Gly Gly Ile His Asp Ala Phe Val
145                 150                 155                 160

Thr Phe Gly Glu Ala Leu Trp Glu Pro Ser Leu Ile Phe Ala Leu Ala
                165                 170                 175

His Phe Asp Gly Ile Leu Gly Leu Gly Phe Pro Thr Leu Ala Val Gly
            180                 185                 190

Gly Val Gln Pro Pro Leu Asp Ala Met Val Glu Gln Gly Leu Leu Glu
        195                 200                 205

Lys Pro Val Phe Ser Phe Tyr Leu Asn Arg Asp Ser Glu Gly Ser Asp
    210                 215                 220

Gly Gly Glu Leu Val Leu Gly Gly Ser Asp Pro Ala His Tyr Val Pro
225                 230                 235                 240

Pro Leu Thr Phe Ile Pro Val Thr Ile Pro Ala Tyr Trp Gln Val His
                245                 250                 255

Met Glu Ser Val Lys Val Gly Thr Gly Leu Ser Leu Cys Ala Gln Gly
            260                 265                 270

Cys Ser Ala Ile Leu Asp Thr Gly Thr Ser Leu Ile Thr Gly Pro Ser
        275                 280                 285

Glu Glu Ile Arg Ala Leu Asn Lys Ala Ile Gly Gly Tyr Pro Phe Leu
    290                 295                 300

Asn Gly Gln Tyr Phe Ile Gln Cys Ser Lys Thr Pro Thr Leu Pro Pro
305                 310                 315                 320

Val Ser Phe His Leu Gly Gly Val Trp Phe Asn Leu Thr Gly Gln Asp
                325                 330                 335

Tyr Val Ile Lys Ile Leu Gln Ser Asp Val Gly Leu Cys Leu Leu Gly
            340                 345                 350

Phe Gln Ala Leu Asp Ile Pro Lys Pro Ala Gly Pro Leu Trp Ile Leu
        355                 360                 365

Gly Asp Val Phe Leu Gly Pro Tyr Val Ala Val Phe Asp Arg Gly Asp
    370                 375                 380
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Lys Asn Val Gly Pro Arg Val Gly Leu Ala Arg Ala Gln Ser Arg Ser
385 390 395 400

Thr Asp Arg Ala Glu Arg Arg Thr Thr Gln Ala Gln Phe Phe Lys Arg
405 410 415

Arg Pro Gly

<210> 45
<211> 3338
<212> DNA
<213> Mus musculus

<400> 45
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<210> 46
<211> 221
<212> PRT
<213> Mus musculus

<400> 46
Met Ala Phe Val Lys Ser Gly Trp Leu Leu Arg Gln Ser Thr Ile Leu
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Lys Arg Trp Lys Lys Asn Trp Phe Asp Leu Trp Ser Asp Gly His Leu
             20                  25                  30

Ile Tyr Tyr Asp Asp Gln Thr Arg Gln Ser Ile Glu Asp Lys Val His
         35                  40                  45

Met Pro Val Asp Cys Ile Asn Ile Arg Thr Gly His Glu Cys Arg Asp
     50                  55                  60

Ile Gln Pro Pro Asp Gly Lys Pro Arg Asp Cys Leu Leu Gln Ile Val
 65                  70                  75                  80

Cys Arg Asp Gly Lys Thr Ile Ser Leu Cys Ala Glu Ser Thr Asp Asp
                 85                  90                  95

Cys Leu Ala Trp Lys Phe Thr Leu Gln Asp Ser Arg Thr Asn Thr Ala
            100                 105                 110

Tyr Val Gly Ser Ala Ile Leu Ser Glu Glu Thr Ala Val Ala Ala Ser
        115                 120                 125

Pro Pro Pro Tyr Ala Thr Tyr Ala Thr Xaa Thr Pro Glu Val Tyr Gly
    130                 135                 140

Tyr Gly Pro Tyr Ser Gly Ala Tyr Pro Ala Gly Thr Gln Val Val Tyr
145                 150                 155                 160

Ala Ala Asn Gly Gln Ala Tyr Ala Val Pro Tyr Gln Tyr Pro Tyr Ala
                165                 170                 175

Gly Val Tyr Gly Gln Gln Pro Ala Asn Gln Val Ile Ile Arg Glu Arg
            180                 185                 190

Tyr Arg Asp Asn Asp Ser Asp Leu Ala Leu Gly Met Leu Ala Gly Ala
        195                 200                 205

Ala Thr Gly Met Ala Leu Gly Ser Leu Phe Trp Val Phe
    210                 215                 220


<210> 47
<211> 2396
<212> DNA
<213> Mus musculus
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<400> 47
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<211> 693
<212> PRT
<213> Mus musculus

<400> 48
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Leu Met Tyr Leu Asn Thr Leu Ala Gly Arg Thr Tyr Asn Asp Tyr Met
35 40 45

Gln Tyr Pro Val Phe Pro Trp Val Leu Ala Asp Tyr Thr Ser Glu Met
50 55 60

Leu Asn Leu Thr Asn Pro Lys Thr Phe Arg Asp Leu Ser Lys Pro Met
65 70 75 80

Gly Ala Gln Thr Lys Glu Arg Lys Leu Lys Phe Thr Gln Arg Phe Lys

8 90

Asp Val Glu Lys Ile Glu Gly Asp Met Thr Val Gln Cys His Tyr Tyr
100 105 110

Thr His Tyr Ser Ser Ala Ile Ile Val Ala Ser Tyr Leu Val Arg Met
115 120 125

Pro Pro Phe Thr Gln Ala Phe Cys Ser Leu Gln Gly Gly Ser Phe Asp
130 135 140

Val Ala Asp Arg Met Phe His Ser Val Lys Ser Thr Trp Glu Ser Ala
145 150 155 160

Ser Lys Glu Asn Met Ser Asp Val Arg Glu Leu Thr Pro Glu Phe Phe
165 170 175

Tyr Leu Pro Glu Phe Leu Thr Asn Cys Asn Ala Val Glu Phe Gly Cys
180 185 190

Met Gln Asp Gly Thr Thr Leu Gly Asp Val Gln Leu Pro Pro Trp Ala
195 200 205

Asp Gly Asp Pro Arg Lys Phe Ile Ser Leu His Arg Gln Ala Leu Glu
210 215 220

Ser Asp Phe Val Ser Ser Asn Leu His His Trp Ile Asp Leu Ile Phe
225 230 235 240

Gly Tyr Lys Gln Gln Gly Pro Ala Ala Val Glu Ala Val Asn Thr Phe
245 250 255

His Pro Tyr Phe Tyr Gly Asp Arg Ile Asp Leu Gly Ser Ile Thr Asp
260 265 270

Pro Leu Ile Lys Ser Thr Ile Leu Gly Phe Ile Ser Asn Phe Gly Gln
275 280 285

Val Pro Lys Gln Ile Phe Thr Lys Pro His Pro Ser Arg Asn Thr Thr
290 295 300

Gly Lys Asn Pro Gly Pro Gly Lys Asp Ala Ser Thr Pro Val Gly Leu
305 310 315 320

Pro Gly His Ser Gln Ser Phe Leu His Ser Leu Pro Ala Leu Arg Pro
325 330 335

Ser Gln Val Thr Val Lys Asp Met Tyr Leu Phe Ser Leu Gly Ser Glu
340 345 350

Ser Pro Lys Gly Ala Ile Gly His Ile Val Pro Thr Glu Lys Ser Ile
355 360 365

Leu Ala Val Glu Lys Asn Lys Leu Leu Met Pro Pro Leu Trp Asn Arg
370 375 380

Thr Phe Ser Trp Gly Phe Asp Asp Phe Ser Cys Cys Leu Gly Ser Tyr
385 390 395 400

Gly Ser Asp Lys Ile Leu Met Thr Phe Glu Asn Leu Ala Ala Trp Gly
405 410 415

Pro Cys Leu Cys Ala Val Cys Pro Ser Pro Thr Met Ile Val Thr Ser
420 425 430

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Gly Ala Ser Ala Val Val Cys Ile Trp Glu Leu Ser Leu Val Lys Gly
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Arg Pro Arg Gly Leu Lys Leu Arg Gln Ala Leu Tyr Gly His Thr Gln
    450                 455                 460

Ala Val Thr Cys Leu Thr Ala Ser Val Thr Phe Ser Leu Leu Val Ser
465                 470                 475                 480

Gly Ser Gln Asp Arg Thr Cys Ile Leu Trp Asp Leu Asp His Leu Ser
                485                 490                 495

Arg Val Ala Cys Leu Pro Val His Arg Glu Gly Ile Ser Ala Ile Ala
            500                 505                 510

Ile Ser Asp Val Ser Gly Thr Ile Val Ser Cys Ala Gly Ala His Leu
        515                 520                 525

Ser Leu Trp Asn Val Asn Gly Gln Pro Leu Ala Ser Ile Thr Thr Ala
    530                 535                 540

Trp Gly Pro Glu Gly Thr Ile Thr Cys Cys Cys Ile Val Glu Gly Pro
545                 550                 555                 560

Ala Trp Asp Ala Ser His Val Ile Ile Thr Gly Ser Lys Asp Gly Met
                565                 570                 575

Val Arg Ile Trp Lys Thr Glu Asp Val Lys Met Pro Val Pro Arg Gln
            580                 585                 590

Ala Val Met Glu Glu Pro Ser Thr Glu Pro Leu Ser Pro Arg Gly His
        595                 600                 605

Lys Trp Ala Lys Asn Leu Ala Leu Ser Arg Glu Leu Asp Val Ser Val
    610                 615                 620

Ala Leu Ser Gly Lys Pro Ser Lys Ala Ser Pro Ala Val Thr Ala Leu
625                 630                 635                 640

Ala Ile Thr Arg Asn Gln Ser Lys Leu Leu Val Gly Asp Glu Lys Gly
                645                 650                 655

Glu Ser Ser Ala Gly Leu Leu Met Gly Arg Arg Gln Gly Ala Gly Gly
            660                 665                 670

Thr Asp Leu Lys Ala Trp Ser Pro Gly Val Gly Ser Asn Arg Leu Gln
        675                 680                 685

Ala Gln Asp Asp Val
    690
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<211> 2363
<212> DNA
<213> Mus musculus

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catctcagtc acatggaacg gaccctgtct cacagtcaaa agctctcaga tggagacctg 240
gtaagatggg catctggagg gctggtcctg cagggaattt ataagaccaa ccacccaaga 300

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tttacacaga ctatagagag ggtgggtttc agttcaaccc ccttagttaa aggagaaggt 480
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<210> 50
<211> 778
<212> PRT
<213> Mus musculus

<400> 50

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Glu Gly Lys His Xaa Gln Glu Glu Ala Val Arg Arg Lys Gly Ala Xaa
             20                  25                  30

Leu Arg Gln Ala Met Glu Ile Pro Glu Glu Cys Trp Pro Thr Ala Glu
         35                  40                  45

Val Ser Leu Lys Asp Ile Thr Glu Ile Met Glu Arg His Leu Ser His
     50                  55                  60

Met Glu Arg Thr Leu Ser His Ser Gln Lys Leu Ser Asp Gly Asp Leu
 65                  70                  75                  80

Val Arg Trp Ala Ser Gly Gly Leu Val Leu Gln Gly Ile Tyr Lys Thr
                 85                  90                  95

Asn His Pro Arg Ser Leu Ile Gln Lys Arg Glu Glu Leu Leu Ser Val
            100                 105                 110

Pro Lys Gln Phe Ser Leu Ala Gly Pro Val His Gly Thr Glu Ile Lys
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        115                 120                 125

Thr Lys Glu Phe Ser Ser Phe Gln Glu Gln Ala Met Phe Thr Gln Thr
    130                 135                 140

Ile Glu Arg Val Gly Phe Ser Ser Thr Pro Leu Val Lys Gly Glu Gly
145                 150                 155                 160

Trp Gly Leu Ser Leu Glu Ala Gly Met Gly His Asn Lys Gln Thr Glu
                165                 170                 175

Ser Glu Asp Asn Tyr Gln Ser His Ser Lys Gln Thr Tyr Phe Cys Ser
            180                 185                 190

Ala Arg Phe Ser Tyr Ile Pro Leu Ala Thr Cys His Phe His Ile Asn
        195                 200                 205

Asp Leu Glu Leu Ser Gln Ala Ala Leu Gln Glu Leu Lys Ser Ile Glu
    210                 215                 220

Glu Ile Leu Glu Gln Thr Thr His His Arg Asp Gly Leu Pro Leu Leu
225                 230                 235                 240

Arg His Arg Ala Lys Asn Phe Phe His Arg Phe Gly Ser His Ala Asn
                245                 250                 255

Gln Gly Pro Val His Leu Gly Gly Ile Tyr Cys Trp Lys Ala Ile Ser
            260                 265                 270

Glu Gly Phe Lys Ser Glu His Leu Ala Asp Val Lys Gln Gln Ala Glu
        275                 280                 285

Glu Ser Leu Asn Ile Tyr Ile Met Gly Ser Tyr Ser Gly Phe Gly Val
    290                 295                 300

Lys Val Gly Ala Ser Val Asn Ile Thr Asn Ser Lys Ser Lys Thr Ala
305                 310                 315                 320

Phe Tyr Ser Lys Thr His Leu Asn Ser Gln Thr Lys Val Gln Leu Ser
                325                 330                 335

Val Ala Lys Ile Gly Gly Pro Ala Glu Ala Asp Gly Ile Ala Gln Trp
            340                 345                 350

Thr Ala Gly Leu Val Ala Ser Asn Gln Thr Trp Ser Val Ile Asp Arg
        355                 360                 365

Lys Leu Gln Leu Val Pro Ile Trp Asp Ile Ile Leu Ser Ser His Arg
    370                 375                 380

Thr Glu Phe Lys Asn Ala Leu Gln Leu Ala Asn Cys Leu Lys Asp His
385                 390                 395                 400

Tyr Thr Ala Leu Thr Glu Leu Ala Ala Gln Ile Gln Glu Gly Glu Glu
                405                 410                 415

Phe Leu Thr Ala Arg Lys Glu Ala Lys Leu Phe Leu Lys Asn Val Lys
            420                 425                 430

Gly Trp Glu Val Ser Asp Pro Glu Glu Gln Leu Arg Lys Leu Val Asp
        435                 440                 445

Phe Met Gln Thr Leu Ser Gln Lys Ile Lys Ser Tyr Asp Ile Trp Ile
    450                 455                 460
```

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Asn Thr Cys Leu Ile Asp Trp Asp Leu Gln Asn Phe Leu Ile Asn Ile
465                 470                 475                 480

Val Asn Phe Cys Lys Asn Ser Pro Thr Tyr Lys Thr His Phe Ile Lys
                485                 490                 495

Ser Gln Leu Cys Ser Leu Leu Glu Pro His Val Tyr Lys Val Thr Asn
            500                 505                 510

Phe Pro Gln Ala Gln Ser Ile Ile Gln Trp Ile Asn Gln Ser Glu Ser
        515                 520                 525

Glu Glu Glu Leu Val Lys Ile Thr Ser Phe Ser Glu Phe Ile Asn Thr
    530                 535                 540

Leu Lys Lys Thr His Lys Tyr Leu Met Glu Glu Ser Phe Lys Thr Glu
545                 550                 555                 560

Pro Pro Glu Arg Val Glu Glu Ala Lys Arg Met Ala Thr Tyr Glu Val
                565                 570                 575

Thr Thr Ala Leu Ser Ser Phe Leu Lys Tyr Leu Arg Glu Thr Gln Gln
            580                 585                 590

Pro Asp Met Gln Leu Leu Leu Leu Ser Ile Ala Thr Gly Val Gly Tyr
        595                 600                 605

Gln Leu Val Asn Ser Ile Phe Gln His Leu Leu Gly Cys Asp Glu Leu
    610                 615                 620

Asn Phe Leu Leu Asp Gln Met Glu Asn Asn Glu His Lys Tyr Gln Glu
625                 630                 635                 640

Leu Lys Asn Ile Cys Asn Tyr Arg Ala Gln Ala Phe Leu Val Leu Thr
                645                 650                 655

Ala Leu Arg Ala Thr Val Glu Ile Thr Asp Val Ser Thr Glu Glu Lys
            660                 665                 670

Gly Gln Arg Leu Thr Leu Ile Gln Gln His Met Gly Ser Leu Leu Ser
        675                 680                 685

Glu Glu Val Ala His Val Leu Thr Lys His Gly Glu His His Asp Trp
    690                 695                 700

Glu Arg Leu Glu Asn Asp Leu Arg Leu Leu Ile Glu Gly Asp Tyr Lys
705                 710                 715                 720

Ala Thr Thr His Ser Leu Gln Met Asp Glu Val Lys Lys Gln Leu Gln
                725                 730                 735

Ser Xaa Cys His Glu Lys Lys Gln Thr Tyr Lys Gln Gln Gly Asn Glu
            740                 745                 750

Asn Arg Thr Lys Glu Met Ile Glu Asn Gly His Phe Leu Asp Leu Leu
        755                 760                 765

Gln Arg Leu Gly Leu Asp Asn Tyr Tyr Pro
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<210> 51
<211> 1712

<212> DNA
<213> Mus musculus

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<210> 52
<211> 336
<212> PRT
<213> Mus musculus

<400> 52
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Tyr Tyr Gly Asn Val Leu Lys Thr Ser Ala Asp Leu Gln Thr Asn Ala
20 25 30

Cys Val Thr Arg Ala Lys Pro Val Pro Ser Tyr Ile Arg Glu Ser Leu
35 40 45

Gln Asn Val His Glu Asp Val Ser Ser Arg Tyr Tyr Gly Cys Gly Leu
50 55 60

Thr Val Pro Glu Arg Leu Glu Asn Cys Arg Ile Leu Asp Leu Gly Ser
65 70 75 80

Gly Ser Gly Arg Asp Cys Tyr Val Leu Ser Gln Leu Val Gly Glu Lys
85 90 95

Gly His Val Thr Gly Ile Asp Met Thr Glu Val Gln Val Glu Val Ala
100 105 110

Lys Thr Tyr Leu Glu His His Met Glu Lys Phe Gly Phe Gln Ala Pro
115 120 125

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Asn Val Thr Phe Leu His Gly Arg Ile Glu Lys Leu Ala Glu Ile Gly
    130                 135                 140

Ile Gln Ser Glu Ser Tyr Gly Ile Val Ile Ser Asn Cys Val Ile Asn
145                 150                 155                 160

Leu Val Pro Asp Lys Gln Gln Val Leu Gln Glu Val Tyr Arg Val Leu
                165                 170                 175

Lys His Gly Gly Glu Leu Tyr Phe Ser Asp Val Tyr Ala Ser Leu Glu
            180                 185                 190

Val Pro Glu Asp Ile Lys Ser His Lys Val Leu Trp Gly Glu Cys Leu
        195                 200                 205

Gly Gly Ala Leu Tyr Trp Lys Asp Leu Ala Ile Ile Ala Gln Lys Ile
    210                 215                 220

Gly Phe Cys Pro Pro Arg Leu Val Thr Ala Asp Ile Ile Thr Val Glu
225                 230                 235                 240

Asn Lys Glu Leu Glu Gly Val Leu Gly Asp Cys Arg Phe Val Ser Ala
                245                 250                 255

Thr Phe Arg Leu Phe Lys Leu Pro Lys Thr Glu Pro Ala Glu Arg Cys
            260                 265                 270

Arg Val Val Tyr Asn Gly Gly Ile Lys Gly His Glu Lys Glu Leu Ile
        275                 280                 285

Phe Asp Ala Asn Phe Thr Phe Lys Glu Gly Glu Ala Val Ala Val Asp
    290                 295                 300

Glu Glu Thr Ala Ala Val Leu Lys Asn Ser Arg Phe Ala Pro Asp Phe
305                 310                 315                 320

Leu Phe Thr Pro Val Asp Ala Ser Leu Pro Ala Pro Gln Gly Pro Phe
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<210> 53
<211> 3599
<212> DNA
<213> Mus musculus

<400> 53

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<210> 54
<211> 687
<212> PRT
<213> Mus musculus

<400> 54

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Phe Ser Leu Val Gln Gly Ala His Ser Gly Ser Pro Arg Glu Asp Phe
             20                  25                  30

Arg Phe Cys Gly Gln Arg Asn Gln Thr Gln Gln Ser Thr Leu His Tyr
         35                  40                  45

Asp Gln Ser Ser Glu Pro His Ile Phe Val Trp Asn Thr Glu Glu Thr
     50                  55                  60
```

```
Leu Thr Ile Arg Ala Pro Phe Leu Ala Ala Pro Asp Ile Pro Ala Phe
 65                  70                  75                  80

Phe Pro Glu Pro Arg Gly Leu Tyr His Phe Cys Leu Tyr Trp Ser Arg
                85                  90                  95

His Thr Gly Arg Leu His Leu Arg Tyr Gly Lys His Asp Tyr Leu Leu
            100                 105                 110

Ser Ser Gln Ala Ser Arg Leu Leu Cys Phe Gln Lys Gln Glu Gln Ser
        115                 120                 125

Leu Lys Gln Gly Ala Pro Leu Ile Ala Thr Ser Val Ser Ser Trp Gln
    130                 135                 140

Ile Pro Gln Asn Thr Ser Leu Pro Gly Ala Pro Ser Phe Ile Phe Ser
145                 150                 155                 160

Phe His Asn Ala Pro His Lys Val Ser His Asn Ala Ser Val Asp Met
                165                 170                 175

Cys Asp Leu Lys Lys Glu Leu Gln Gln Leu Ser Arg Tyr Leu Gln His
            180                 185                 190

Pro Gln Lys Ala Ala Lys Arg Pro Thr Ala Ala Phe Ile Ser Gln Gln
        195                 200                 205

Leu Gln Ser Leu Glu Ser Lys Leu Thr Ser Val Ser Phe Leu Gly Asp
    210                 215                 220

Thr Leu Ser Phe Glu Glu Asp Arg Val Asn Ala Thr Val Trp Lys Leu
225                 230                 235                 240

Pro Pro Thr Ala Gly Leu Glu Asp Leu His Ile His Ser Gln Lys Glu
                245                 250                 255

Glu Glu Gln Ser Glu Val Gln Ala Tyr Ser Leu Leu Leu Pro Arg Ala
            260                 265                 270

Val Phe Gln Gln Thr Arg Gly Arg Arg Arg Asp Asp Ala Lys Arg Leu
        275                 280                 285

Leu Val Val Asp Phe Ser Ser Gln Ala Leu Phe Gln Asp Lys Asn Ser
    290                 295                 300

Ser Gln Val Leu Gly Glu Lys Val Leu Gly Ile Val Val Gln Asn Thr
305                 310                 315                 320

Lys Val Thr Asn Leu Ser Asp Pro Val Val Leu Thr Phe Gln His Gln
                325                 330                 335

Pro Gln Pro Lys Asn Val Thr Leu Gln Cys Val Phe Trp Val Glu Asp
            340                 345                 350

Pro Ala Ser Ser Ser Thr Gly Ser Trp Ser Ser Ala Gly Cys Glu Thr
        355                 360                 365

Val Ser Arg Asp Thr Gln Thr Ser Cys Leu Cys Asn His Leu Thr Tyr
    370                 375                 380

Phe Ala Val Leu Met Val Ser Ser Thr Glu Val Glu Ala Thr His Lys
385                 390                 395                 400

His Tyr Leu Thr Leu Leu Ser Tyr Val Gly Cys Val Ile Ser Ala Leu
```

```
                405                 410                 4

Ala Cys Val Phe Thr Ile Ala Ala Tyr Leu Cys Ser Arg Arg Lys Ser
            420                 425                 430

Arg Asp Tyr Thr Ile Lys Val His Met Asn Leu Leu Ser Ala Val Phe
        435                 440                 445

Leu Leu Asp Val Ser Phe Leu Leu Ser Glu Pro Val Ala Leu Thr Gly
    450                 455                 460

Ser Glu Ala Ala Cys Arg Thr Ser Ala Met Phe Leu His Phe Ser Leu
465                 470                 475                 480

Leu Ala Cys Leu Ser Trp Met Gly Leu Glu Gly Tyr Asn Leu Tyr Arg
                485                 490                 495

Leu Val Val Glu Val Phe Gly Thr Tyr Val Pro Gly Tyr Leu Leu Lys
            500                 505                 510

Leu Ser Ile Val Gly Trp Gly Phe Pro Val Phe Leu Val Thr Leu Val
        515                 520                 525

Ala Leu Val Asp Val Asn Asn Tyr Gly Pro Ile Ile Leu Ala Val Arg
    530                 535                 540

Arg Thr Pro Glu Arg Val Thr Tyr Pro Ser Met Cys Trp Ile Arg Asp
545                 550                 555                 560

Ser Leu Val Ser Tyr Val Thr Asn Leu Gly Leu Phe Ser Leu Val Phe
                565                 570                 575

Leu Phe Asn Leu Ala Met Leu Ala Thr Met Val Val Gln Ile Leu Arg
            580                 585                 590

Leu Arg Pro His Ser Gln Asn Trp Pro His Val Leu Thr Leu Leu Gly
        595                 600                 605

Leu Ser Leu Val Leu Gly Leu Pro Trp Ala Leu Val Phe Phe Ser Phe
    610                 615                 620

Ala Ser Gly Thr Phe Gln Leu Val Ile Leu Tyr Leu Phe Ser Ile Ile
625                 630                 635                 640

Thr Ser Tyr Gln Gly Phe Leu Ile Phe Leu Trp Tyr Trp Ser Met Arg
                645                 650                 655

Phe Gln Ala Gln Gly Gly Pro Ser Pro Leu Lys Asn Asn Ser Asp Ser
            660                 665                 670

Ala Lys Leu Pro Ile Ser Ser Gly Ser Thr Ser Ser Ser Arg Ile
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<210> 55
<211> 3590
<212> DNA
<213> Mus musculus

<400> 55
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atggaagtgg ctgaaagaaa cttctcgccc tgctgattct gagccccgcg tcgtgccgcg 180
cgccctcaat tacctcatcg acctgtggtc ttgacagaac attcttcaca atccaaaaga 240

```
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catcaagaat aatggacaag tctacacata cccagatggt aaatctggca tggctacctg 420
tgagatgtgt gggatggtcg gtgtgcgaga tgctttttac tctaaaacga aacgtttctg 480
cagtgtttcc tgttcaagaa gttactcgtc aaactctaag aaggcaagca ttctggccag 540
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<210> 56
<211> 631
<212> PRT
<213> Mus musculus

<400> 56
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Pro Ile Ile Lys Asn Asn Gly Gln Val Tyr Thr Tyr Pro Asp Gly Lys
        35                  40                  45
Ser Gly Met Ala Thr Cys Glu Met Cys Gly Met Val Gly Val Arg Asp
    50                  55                  60
Ala Phe Tyr Ser Lys Thr Lys Arg Phe Cys Ser Val Ser Cys Ser Arg
65                  70                  75                  80
Ser Tyr Ser Ser Asn Ser Lys Lys Ala Ser Ile Leu Ala Arg Leu Gln
                85                  90                  95
Gly Lys Pro Pro Thr Lys Lys Ala Lys Val Leu Gln Lys Gln Pro Leu
            100                 105                 110
Val Ala Lys Leu Ala Ala Tyr Ala Gln Tyr Gln Ala Thr Leu Gln Asn
        115                 120                 125
Gln Ala Lys Thr Lys Ala Gly Asn Ser Ala Ile Ser Val Glu Gly Phe
    130                 135                 140
Ser Trp Gly Asn Tyr Ile Asn Ser Asn Ser Phe Ile Ala Ala Pro Val
145                 150                 155                 160
Ala Cys Phe Lys His Ala Pro Met Gly Thr Cys Trp Gly Asp Ile Ser
                165                 170                 175
Glu Asn Val Arg Ile Glu Val Pro Asn Thr Asp Cys Ser Leu Pro Thr
            180                 185                 190
Lys Val Phe Trp Ile Ala Gly Ile Ile Lys Leu Ala Gly Tyr Asn Ala
        195                 200                 205
Leu Leu Arg Tyr Glu Gly Phe Glu Asn Asp Ser Ser Leu Asp Phe Trp
    210                 215                 220
Cys Asn Ile Cys Gly Ser Asp Ile His Pro Val Gly Trp Cys Ala Ala
225                 230                 235                 240
Ser Gly Lys Pro Leu Val Pro Pro Arg Thr Val Gln His Lys Tyr Thr
                245                 250                 255
Asn Trp Lys Ala Phe Leu Val Lys Arg Leu Thr Gly Ala Lys Thr Leu
            260                 265                 270
Pro Pro Asp Phe Ser Gln Lys Val Ser Glu Ser Met Gln Tyr Pro Phe
        275                 280                 285
Lys Pro Cys Met Arg Val Glu Val Val Asp Lys Arg His Leu Cys Arg
    290                 295                 300
Thr Arg Val Ala Val Val Glu Ser Val Ile Gly Gly Arg Leu Arg Leu
305                 310                 315                 320
Val Tyr Glu Glu Ser Glu Asp Gly Thr Asp Asp Phe Trp Cys His Met
                325                 330                 335
His Ser Pro Leu Ile His His Ile Gly Trp Ser Arg Ser Ile Gly His
            340                 345                 350
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Arg Phe Lys Arg Ser Asp Ile Thr Lys Lys Gln Asp Gly His Phe Asp
        355                 360                 365

Thr Pro Pro His Leu Phe Ala Lys Val Lys Glu Val Asp Gln Ser Gly
    370                 375                 380

Glu Trp Phe Lys Glu Gly Met Lys Leu Glu Ala Ile Asp Pro Leu Asn
385                 390                 395                 400

Leu Ser Thr Ile Cys Val Ala Thr Ile Arg Lys Val Leu Ala Asp Gly
                405                 410                 415

Phe Leu Met Ile Gly Ile Asp Gly Ser Glu Ala Ala Asp Gly Ser Asp
            420                 425                 430

Trp Phe Cys Tyr His Ala Thr Ser Pro Ser Ile Phe Pro Val Gly Phe
        435                 440                 445

Cys Glu Ile Asn Met Ile Glu Leu Thr Pro Pro Arg Gly Tyr Thr Lys
    450                 455                 460

Leu Pro Phe Lys Trp Phe Asp Tyr Leu Arg Glu Thr Gly Ser Ile Ala
465                 470                 475                 480

Ala Pro Val Lys Leu Phe Asn Lys Asp Val Pro Asn His Gly Phe Arg
                485                 490                 495

Val Gly Met Lys Leu Glu Ala Val Asp Leu Met Glu Pro Arg Leu Ile
            500                 505                 510

Cys Val Ala Thr Val Thr Arg Ile Ile His Arg Leu Leu Arg Ile His
        515                 520                 525

Phe Asp Gly Trp Glu Glu Glu Tyr Asp Gln Trp Val Asp Cys Glu Ser
    530                 535                 540

Pro Asp Leu Asn Pro Val Gly Trp Cys Gln Leu Thr Gly Tyr Gln Leu
545                 550                 555                 560

Gln Pro Pro Ala Ser Gln Ser Ser Arg Glu Ser Gln Ser Ala Ser Ser
                565                 570                 575

Lys Gln Lys Lys Lys Ala Lys Ser Gln Gln Tyr Lys Gly His Lys Lys
            580                 585                 590

Met Thr Thr Ser Gln Leu Lys Glu Glu Leu Leu Asp Gly Glu Asp Tyr
        595                 600                 605

Ser Phe Leu His Gly Ala Ser Asp Gln Glu Ser Asn Gly Ser Ala Thr
    610                 615                 620

Val Tyr Ile Lys Gln Glu Pro
625                 630
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<210> 57
<211> 1650
<212> DNA
<213> Mus musculus

<400> 57
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<210> 58
<211> 359
<212> PRT
<213> Mus musculus

<400> 58

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Met Glu Ser Val Glu Pro Pro Val Lys Asp Gly Ile Leu Tyr Gln Gln
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His Val Lys Phe Gly Lys Lys Cys Trp Arg Lys Val Trp Ala Leu Leu
            20                  25                  30

Tyr Ala Gly Gly Pro Ser Gly Val Ala Arg Leu Glu Ser Trp Asp Val
         35                  40                  45

Arg Asp Gly Gly Leu Gly Pro Ala Gly Asp Arg Ser Thr Gly Pro Ser
     50                  55                  60

Arg Arg Gly Glu Arg Arg Val Ile Arg Leu Ala Asp Cys Val Ser Val
 65                  70                  75                  80

Leu Pro Ala Asp Gly Glu Ser Cys Pro Arg Asp Thr Gly Ala Phe Leu
                85                  90                  95

Ile Thr Thr Thr Glu Arg Ser His Leu Leu Ala Ala Gln His Arg Gln
            100                 105                 110

Ser Trp Val Asp Pro Ile Cys Gln Leu Ala Phe Pro Gly Thr Gly Glu
        115                 120                 125

Cys Ser Ser Gly Ser Gly Gln Ala Glu Asn Pro Lys Arg Gly Phe Val
    130                 135                 140

Pro Met Glu Glu Asn Ser Ile Tyr Ser Ser Trp Gln Glu Val Thr Glu
145                 150                 155                 160

Phe Pro Val Ile Val Gln Lys Thr Glu Ala Thr Ser Arg Cys Gln Leu
```

```
                165                 170                 1

Lys Gly Pro Tyr Leu Leu Val Leu Gly Gln Asp Asp Ile Gln Leu Arg
            180                 185                 190

Glu Thr Ser Lys Pro Gln Ala Cys Phe Ser Trp Pro Tyr Arg Phe Leu
        195                 200                 205

Arg Lys Tyr Gly Ser Asp Lys Gly Val Phe Ser Phe Glu Ala Gly Arg
    210                 215                 220

Arg Cys Asp Ser Gly Glu Gly Leu Phe Ala Phe Ser Ser Pro Arg Ala
225                 230                 235                 240

Pro Asp Ile Cys Gly Val Val Ala Ala Ala Ile Ala Arg Gln Arg Glu
                245                 250                 255

Arg Leu Pro Glu Leu Ala Met Ser Pro Pro Cys Pro Leu Pro Arg Ala
            260                 265                 270

Leu Ser Leu Pro Ser Leu Glu Pro Pro Gly Glu Leu Arg Glu Val Ala
        275                 280                 285

Pro Gly Phe Glu Leu Pro Thr Pro Arg Lys Leu Pro Leu Thr Asp Pro
    290                 295                 300

Gly Pro Gln Ser Leu Pro Leu Leu Leu Ser Pro Asn Thr Arg Arg Thr
305                 310                 315                 320

Gly Ile Arg Ser Leu Cys Val Arg Val Gln Ala Asp Gln Gln Ala His
                325                 330                 335

Arg His Gly Gly Ala Phe Leu Glu Arg Val His Ala Gly Gly Gln Pro
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Trp Ala Asp Gln Trp Gly Ser
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<210> 59
<211> 1750
<212> DNA
<213> Mus musculus

<400> 59

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<211> 439
<212> PRT
<213> Mus musculus

<400> 60
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Ser Ala Ser Val Ser Ser Val Arg Ser Arg Ser Arg Thr Thr Ser Gly
        35                  40                  45

Thr Gly Leu Ser Ser Pro Pro Leu Ala Ala Gln Thr Val Val Pro Leu
    50                  55                  60

Gln His Cys Lys Ile Pro Glu Leu Pro Val Gln Ala Ser Ile Leu Phe
65                  70                  75                  80

Glu Leu Gln Leu Phe Phe Cys Gln Leu Ile Ala Leu Phe Val His Tyr
                85                  90                  95

Ile Asn Ile Tyr Lys Thr Val Trp Trp Tyr Pro Pro Ser His Pro Pro
            100                 105                 110

Ser His Thr Ser Leu Asn Phe His Leu Ile Asp Phe Asn Leu Leu Met
        115                 120                 125

Val Thr Ala Ile Val Leu Gly Arg Arg Phe Ile Gly Ser Ile Val Lys
    130                 135                 140

Glu Ala Ser Gln Arg Gly Lys Gly Leu Pro Leu Pro Leu His Pro Ala
145                 150                 155                 160

Val Pro His Pro Leu His Val Leu Thr Ala Thr Gly Trp Ser Leu Cys
                165                 170                 175

Arg Ser Leu Ile His Leu Phe Arg Thr Tyr Ser Phe Leu Asn Leu Leu
            180                 185                 190

Phe Leu Cys Tyr Pro Phe Gly Met Tyr Ile Pro Phe Leu Gln Leu Asn
        195                 200                 205

Tyr Asp Leu Arg Lys Thr Asn Leu Phe Thr His Met Ala Ser Met Gly
    210                 215                 220

Pro Arg Glu Ala Val Ser Gly Leu Ala Arg Ser Arg Asp Tyr Phe Leu
225                 230                 235                 240

Thr Leu Arg Glu Thr Trp Lys Gln His Thr Arg Gln Leu Tyr Gly Pro
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Glu Ala Met Pro Thr His Ala Cys Cys Leu Ser Pro Ser Leu Ile Arg
            260                 265                 270

Asn Glu Val Glu Phe Leu Lys Met Asp Phe Asn Trp Arg Met Lys Glu
        275                 280                 285

Val Leu Val Ser Ser Met Leu Ser Ala Tyr Tyr Val Ala Phe Val Pro
    290                 295                 300

Val Trp Phe Val Lys Asn Thr His Tyr Tyr Asp Lys Arg Trp Ser Cys
305                 310                 315                 320

Glu Leu Phe Leu Leu Val Ser Ile Ser Thr Ser Val Ile Leu Met Gln
                325                 330                 335

His Leu Leu Pro Ala Ser Tyr Cys Asp Leu Leu His Lys Ala Ala Ala
            340                 345                 350

His Leu Ala Cys Trp Gln Lys Val Asp Pro Ala Leu Cys Ser Asn Val
        355                 360                 365

Leu Gln Pro Pro Trp Thr Glu Glu Cys Met Trp Pro Gln Gly Val Leu
    370                 375                 380

Val Lys His Ser Lys Asn Val Tyr Lys Ala Val Gly His Tyr Asn Val
385                 390                 395                 400

Ala Ser Pro Pro Met Ser Pro Thr Ser Ala Ser Ile Ser Phe Ser Ala
                405                 410                 415

Thr Pro Cys Gly Ser Ser Thr Ser Phe Cys Cys Trp Arg Val Pro Ser
            420                 425                 430

Leu Ser Thr Ser Cys Thr Pro
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<210> 61
<211> 3071
<212> DNA
<213> Mus musculus

<400> 61

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<210> 62
<211> 644
<212> PRT
<213> Mus musculus

<400> 62
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Pro Trp Ala Gly Ala Ala Ala Asp Ser Gln Ala Val Val Cys Glu Gly
            20                  25                  30

Thr Ala Cys Tyr Thr Ala His Trp Gly Lys Leu Ser Ala Ala Glu Ala
         35                  40                  45

Gln His Arg Cys Asn Glu Asn Gly Gly Asn Leu Ala Thr Val Lys Ser
     50                  55                  60

Glu Glu Glu Ala Arg His Val Gln Gln Ala Leu Thr Gln Leu Leu Lys
 65                  70                  75                  80

Thr Lys Ala Pro Leu Glu Ala Lys Met Gly Lys Phe Trp Ile Gly Leu
                 85                  90                  95

Gln Arg Glu Lys Gly Asn Cys Thr Tyr His Asp Leu Pro Met Arg Gly
            100                 105                 110

Phe Ser Trp Val Gly Gly Gly Glu Asp Thr Ala Tyr Ser Asn Trp Tyr
        115                 120                 125
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Lys Ala Ser Lys Se:  er Cys Ile Phe Lys Arg Cys Val Ser L   Ile
    130                 135                 140

Leu Asp Leu Ser Leu Thr Pro His Pro Ser His Leu Pro Lys Trp His
145                 150                 155                 160

Glu Ser Pro Cys Gly Thr Pro Glu Ala Pro Gly Asn Ser Ile Glu Gly
                165                 170                 175

Phe Leu Cys Lys Phe Asn Phe Lys Gly Met Cys Arg Pro Leu Ala Leu
            180                 185                 190

Gly Gly Pro Gly Arg Val Thr Tyr Thr Thr Pro Phe Gln Ala Thr Thr
        195                 200                 205

Ser Ser Leu Glu Ala Val Pro Phe Ala Ser Val Ala Asn Val Ala Cys
    210                 215                 220

Gly Asp Glu Ala Lys Ser Glu Thr His Tyr Phe Leu Cys Asn Glu Lys
225                 230                 235                 240

Thr Pro Gly Ile Phe His Trp Gly Ser Ser Gly Pro Leu Cys Val Ser
                245                 250                 255

Pro Lys Phe Gly Cys Ser Phe Asn Asn Gly Gly Cys Gln Gln Asp Cys
            260                 265                 270

Phe Glu Gly Gly Asp Gly Ser Phe Arg Cys Gly Cys Arg Pro Gly Phe
        275                 280                 285

Arg Leu Leu Asp Asp Leu Val Thr Cys Ala Ser Arg Asn Pro Cys Ser
    290                 295                 300

Ser Asn Pro Cys Thr Gly Gly Gly Met Cys His Ser Val Pro Leu Ser
305                 310                 315                 320

Glu Asn Tyr Thr Cys Arg Cys Pro Ser Gly Tyr Gln Leu Asp Ser Ser
                325                 330                 335

Gln Val His Cys Val Asp Ile Asp Glu Cys Gln Asp Ser Pro Cys Ala
            340                 345                 350

Gln Asp Cys Val Asn Thr Leu Gly Ser Phe His Cys Glu Cys Trp Val
        355                 360                 365

Gly Tyr Gln Pro Ser Gly Pro Lys Glu Glu Ala Cys Glu Asp Val Asp
    370                 375                 380

Glu Cys Ala Ala Ala Asn Ser Pro Cys Ala Gln Gly Cys Ile Asn Thr
385                 390                 395                 400

Asp Gly Ser Phe Tyr Cys Ser Cys Lys Glu Gly Tyr Ile Val Ser Gly
                405                 410                 415

Glu Asp Ser Thr Gln Cys Glu Asp Ile Asp Glu Cys Ser Asp Ala Arg
            420                 425                 430

Gly Asn Pro Cys Asp Ser Leu Cys Phe Asn Thr Asp Gly Ser Phe Arg
        435                 440                 445

Cys Gly Cys Pro Pro Gly Trp Glu Leu Ala Pro Asn Gly Val Phe Cys
    450                 455                 460

Ser Arg Gly Thr Val Phe Ser Glu Leu Pro Ala Arg Pro Pro Gln Lys
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465                 70                  475                 480

Glu Asp Asn Asp Asp Arg Lys Glu Ser Thr Met Pro Pro Thr Glu Met
                485                 490                 495

Pro Ser Ser Pro Ser Gly Ser Lys Asp Val Ser Asn Arg Ala Gln Thr
            500                 505                 510

Thr Gly Leu Phe Val Gln Ser Asp Ile Pro Thr Ala Ser Val Pro Leu
        515                 520                 525

Glu Ile Glu Ile Pro Ser Glu Val Ser Asp Val Trp Phe Glu Leu Gly
    530                 535                 540

Thr Tyr Leu Pro Thr Thr Ser Gly His Ser Lys Pro Thr His Glu Asp
545                 550                 555                 560

Ser Val Ser Ala His Ser Asp Thr Asp Gly Gln Asn Leu Leu Leu Phe
                565                 570                 575

Tyr Ile Leu Gly Thr Val Val Ala Ile Ser Leu Leu Leu Val Leu Ala
            580                 585                 590

Leu Gly Ile Leu Ile Tyr His Lys Arg Arg Ala Lys Lys Glu Glu Ile
        595                 600                 605

Lys Glu Lys Lys Pro Gln Asn Ala Ala Asp Ser Tyr Ser Trp Val Pro
    610                 615                 620

Glu Arg Ala Glu Ser Gln Ala Pro Glu Asn Gln Tyr Ser Pro Thr Pro
625                 630                 635                 640

Gly Thr Asp Cys
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<210> 63
<211> 499
<212> DNA
<213> Mus musculus

<400> 63
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atgtgttctg cttgaaaaa 499

<210> 64
<211> 89
<212> PRT
<213> Mus musculus

<400> 64
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Met Arg Thr Ala Pro Ala Ala Ala Ile Leu Cys Glu Leu Gly Leu Leu
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Tyr Gly Asn Gly Gly Val Arg Lys Cys Gly Asn His Lys Arg Arg Gln
             20                  25                  30
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Asn Gln Lys Thr Glu Gly Ser Trp Ala Met Ser His Ser Lys Glu Ser
35 40 45

Leu Pro Pro Asn Ile Cys Thr Gly Leu Leu Gln Glu Gln Glu Met Asn
50 55 60

Tyr Arg Val Gln Pro Leu Arg Leu Gly Leu Asn Met Ile Phe Thr Leu
65 70 75 80

Ser Thr Leu Pro Asn Arg Ala Glu Met
85

<210> 65
<211> 3857
<212> DNA
<213> Mus musculus

<400> 65
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<210> 66
<211> 547
<212> PRT
<213> Mus musculus

<400> 66

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Ser Cys Ser Gly Asp Ser Ile Gln Asp Ser Tyr Thr Gly Met Glu Asn
             20                  25                  30

Ser Asp Lys Asp Ala Met Asn Ser Gln Phe Ala Asn Glu Asp Ala Glu
         35                  40                  45

Ser Gln Lys Phe Leu Thr Asn Gly Phe Leu Gly Lys Lys Lys Leu Ala
     50                  55                  60

Asp Tyr Ala Asp Glu His His Pro Gly Met Thr Ser Phe Gly Met Ser
 65                  70                  75                  80

Ser Phe Asn Leu Ser Asn Ala Ile Met Gly Ser Gly Ile Leu Gly Leu
                 85                  90                  95

Ser Tyr Ala Met Ala Asn Thr Gly Ile Ile Leu Phe Ile Ile Met Leu
            100                 105                 110

Leu Thr Val Ala Ile Leu Ser Leu Tyr Ser Val His Leu Leu Leu Lys
        115                 120                 125

Thr Ala Lys Glu Gly Gly Ser Leu Ile Tyr Glu Lys Leu Gly Glu Lys
    130                 135                 140

Ala Phe Gly Trp Pro Gly Lys Ile Gly Ala Phe Ile Ser Ile Thr Met
145                 150                 155                 160

Gln Asn Ile Gly Ala Met Ser Ser Tyr Leu Phe Ile Ile Lys Tyr Glu
                165                 170                 175

Leu Pro Glu Val Ile Arg Ala Phe Met Gly Leu Glu Glu Asn Thr Gly
            180                 185                 190

Glu Trp Tyr Leu Asn Gly Asn Tyr Leu Val Leu Phe Val Ser Val Gly
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        195                 200                 205

Ile Ile Leu Pro Leu Ser Leu Leu Lys Asn Leu Gly Tyr Leu Gly Tyr
    210                 215                 220

Thr Ser Gly Phe Ser Leu Ser Cys Met Val Phe Phe Val Ser Val Val
225                 230                 235                 240

Ile Tyr Lys Lys Phe Gln Ile Pro Cys Pro Leu Pro Ala Leu Asp His
                245                 250                 255

Asn Asn Gly Asn Leu Thr Phe Asn Asn Thr Leu Pro Ile His Met Ile
            260                 265                 270

Ser Leu Pro Asn Asp Ser Glu Ser Ser Gly Val Asn Phe Met Met Asp
        275                 280                 285

Tyr Ala His His Asn Pro Ala Gly Leu Asp Glu Lys Gln Val Ala Gly
    290                 295                 300

Pro Leu His Ser Asn Gly Val Glu Tyr Glu Ala Gln Gly Ala Glu Lys
305                 310                 315                 320

Cys Gln Pro Lys Tyr Phe Val Phe Asn Ser Arg Thr Ala Tyr Ala Ile
                325                 330                 335

Pro Ile Leu Ala Phe Ala Phe Val Cys His Pro Glu Val Leu Pro Ile
            340                 345                 350

Tyr Ser Glu Leu Lys Asp Arg Ser Arg Arg Lys Met Gln Thr Val Ser
        355                 360                 365

Asn Ile Ser Ile Ser Gly Met Leu Val Met Tyr Leu Leu Ala Ala Leu
    370                 375                 380

Phe Gly Tyr Leu Ser Phe Tyr Gly Asp Val Glu Asp Glu Leu Leu His
385                 390                 395                 400

Ala Tyr Ser Lys Val Tyr Thr Phe Asp Thr Ala Leu Leu Met Val Arg
                405                 410                 415

Leu Ala Val Leu Val Ala Val Thr Leu Thr Val Pro Ile Val Leu Phe
            420                 425                 430

Pro Ile Arg Thr Ser Val Ile Thr Leu Leu Phe Pro Arg Lys Pro Phe
        435                 440                 445

Ser Trp Leu Lys His Phe Gly Ile Ala Ala Ile Ile Ile Ala Leu Asn
    450                 455                 460

Asn Ile Leu Val Ile Leu Val Pro Thr Ile Lys Tyr Ile Phe Gly Phe
465                 470                 475                 480

Ile Gly Ala Ser Ser Ala Thr Met Leu Ile Phe Ile Leu Pro Ala Ala
                485                 490                 495

Phe Tyr Leu Lys Leu Val Lys Lys Glu Pro Leu Arg Ser Pro Gln Lys
            500                 505                 510

Ile Gly Ala Leu Val Phe Leu Val Thr Gly Ile Ile Phe Met Met Gly
        515                 520                 525

Ser Met Ala Leu Ile Ile Leu Asp Trp Ile Tyr Asn Pro Pro Asn Pro
    530                 535                 540
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Asn His His
545

<210> 67
<211> 1070
<212> DNA
<213> Mus musculus

<400> 67
agcgacatgg ccccgcccgc gctccaggcc cagcctccag gcggctctca actgaggttc 60
ctgctgttcc tgctgctgtt gctgctgctg ctgtcatggc catcgcaggg ggacgccctg 120
gcaatgcctg aacagcgacc ctccggccct gagtcccaac tcaacgccga cgagctacgg 180
ggtcgcttcc aggacctgct gagccggctg catgccaacc agagccgaga ggactcgaac 240
tcagaaccaa gtcctgaccc agctgtccgg atactcagtc cagaggtgag attggggtcc 300
cacggccagc tgctactccg cgtcaaccgg gcgtcgctga gtcagggtct ccccgaagcc 360
taccgcgtgc accgagcgct gctcctgctg acgccgacgg cccgcccctg ggacatcact 420
aggcccctga agcgtgcgct cagcctccgg ggaccccgtg ctcccgcatt acgcctgcgc 480
ctgacgccgc ctccggacct ggctatgctg ccctctggcg gcacgcagct ggaactgcgc 540
ttacgggtag ccgccggcag ggggcgccga agcgcgcatg cgcacccaag agactcgtgc 600
ccactgggtc cggggcgctg ctgtcacttg gagactgtgc aggcaactct tgaagacttg 660
ggctggagcg actgggtgct gtccccgcgc cagctgcagc tgagcatgtg cgtgggcgag 720
tgtccccacc tgtatcgctc cgcgaacacg catgcgcaga tcaaagcacg cctgcatggc 780
ctgcagcctg acaaggtgcc tgccccgtgc tgtgtccccc ccagctacac cccggtggtt 840
cttatgcaca ggacagacag tggtgtgtca ctgcagactt atgatgacct ggtggcccgg 900
ggctgccact gcgcttgagc accgggccct gctcctcacc tacactcccc ttcaaggatg 960
ctatttatat ttgtatttat taatattatt aatttattgg ggtcgggctg ggtggatgga 1020
ttgtgtattt atttaaaact ctgctaataa aggtgagctt ggtttcaaaa 1070

<210> 68
<211> 298
<212> PRT
<213> Mus musculus

<400> 68
Met Ala Pro Pro Ala Leu Gln Ala Gln Pro Pro Gly Gly Ser Gln Leu
1 5 10 15

Arg Phe Leu Leu Phe Leu Leu Leu Leu Leu Leu Leu Leu Ser Trp Pro
20 25 30

Ser Gln Gly Asp Ala Leu Ala Met Pro Glu Gln Arg Pro Ser Gly Pro
35 40 45

Glu Ser Gln Leu Asn Ala Asp Glu Leu Arg Gly Arg Phe Gln Asp Leu
50 55 60

Leu Ser Arg Leu His Ala Asn Gln Ser Arg Glu Asp Ser Asn Ser Glu
65 70 75 80

Pro Ser Pro Asp Pro Ala Val Arg Ile Leu Ser Pro Glu Val Arg Leu
85 90 95

Gly Ser His Gly Gln Leu Leu Leu Arg Val Asn Arg Ala Ser Leu Ser
100 105 110

Gln Gly Leu Pro Glu Ala Tyr Arg Val His Arg Ala Leu Leu Leu Leu
115 120 125

Thr Pro Thr Ala Arg Pro Trp Asp Ile Thr Arg Pro Leu Lys Arg Ala
130 135 140

Leu Ser Leu Arg Gly Pro Arg Ala Pro Ala Leu Arg Leu Arg Leu Thr

145 150 155 160

Pro Pro Pro Asp Leu Ala Met Leu Pro Ser Gly Gly Thr Gln Leu Glu
165 170 175

Leu Arg Leu Arg Val Ala Ala Gly Arg Gly Arg Arg Ser Ala His Ala
180 185 190

His Pro Arg Asp Ser Cys Pro Leu Gly Pro Gly Arg Cys Cys His Leu
195 200 205

Glu Thr Val Gln Ala Thr Leu Glu Asp Leu Gly Trp Ser Asp Trp Val
210 215 220

Leu Ser Pro Arg Gln Leu Gln Leu Ser Met Cys Val Gly Glu Cys Pro
225 230 235 240

His Leu Tyr Arg Ser Ala Asn Thr His Ala Gln Ile Lys Ala Arg Leu
245 250 255

His Gly Leu Gln Pro Asp Lys Val Pro Ala Pro Cys Cys Val Pro Ser
260 265 270

Ser Tyr Thr Pro Val Val Leu Met His Arg Thr Asp Ser Gly Val Ser
275 280 285

Leu Gln Thr Tyr Asp Asp Leu Val Ala Arg
290 295

<210> 69
<211> 134
<212> PRT
<213> Mus musculus

<400> 69
Ala Lys Tyr Val Cys Leu Ala Asn Lys Asn Cys Pro Val Asp Lys Arg
1 5 10 15

Arg Arg Asn Arg Cys Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val
20 25 30

Gly Met Val Lys Glu Val Val Arg Thr Asp Ser Leu Lys Gly Arg Arg
35 40 45

Gly Arg Leu Pro Ser Lys Pro Lys Ser Pro Gln Glu Pro Ser Pro Pro
50 55 60

Ser Pro Pro Phe Gln Ala Asn Pro Asp Tyr Gln Met Ser Gly Asp Asp
65 70 75 80

Thr Gln His Ile Gln Gln Phe Tyr Asp Leu Leu Thr Gly Ser Met Glu
85 90 95

Ile Ile Arg Gly Trp Ala Glu Lys Ile Pro Gly Phe Ala Asp Leu Pro
100 105 110

Lys Ala Asp Gln Asp Leu Leu Phe Glu Ser Ala Phe Leu Glu Leu Phe
115 120 125

Val Leu Arg Leu Ala Tyr
130

<210> 70
<211> 140
<212> PRT
<213> Mus musculus

<400> 70
Ser Gln Thr Arg Gln Gln Gly Pro Leu Arg Ser Ile Met Lys Asp Leu
1 5 10 15

His Ser Asp Asp Asn Glu Glu Glu Ser Asp Glu Val Glu Asp Asn Asp
20 25 30

Asn Asp Ser Glu Met Glu Arg Pro Val Asn Arg Gly Xaa Ser Arg Ser
35 40 45

Arg Arg Val Ser Leu Ser Asp Gly Ser Asp Ser Glu Ser Ser Ser Ala
50 55 60

Ser Ser Pro Leu His His Glu Pro Pro Pro Pro Leu Leu Lys Thr Asn
65 70 75 80

Asn Asn Gln Ile Leu Glu Val Lys Ser Pro Ile Lys Gln Ser Lys Ser
85 90 95

Asp Lys Gln Ile Lys Asn Gly Glu Cys Asp Lys Ala Tyr Leu Asp Glu
100 105 110

Leu Val Glu Leu Pro Arg Xaa Xaa Met Thr Leu Arg Glu Arg His Ile
115 120 125

Leu Gln Gln Ile Val Asn Leu Xaa Glu Glu Thr Gly
130 135 140

<210> 71
<211> 89
<212> PRT
<213> Mus musculus

<400> 71
Met Arg Thr Ala Pro Ala Ala Ala Ile Leu Cys Glu Leu Gly Leu Leu
1 5 10 15

Tyr Gly Asn Gly Gly Val Arg Lys Cys Gly Asn His Lys Arg Arg Gln
20 25 30

Asn Gln Lys Thr Glu Gly Ser Trp Ala Met Ser His Ser Lys Glu Ser
35 40 45

Leu Pro Pro Asn Ile Cys Thr Gly Leu Leu Gln Glu Gln Glu Met Asn
50 55 60

Tyr Arg Val Gln Pro Leu Arg Leu Gly Leu Asn Met Ile Phe Thr Leu
65 70 75 80

Ser Thr Leu Pro Asn Arg Ala Glu Met
85

<210> 72
<211> 3121
<212> DNA
<213> Homo sapiens

<400> 72
gaaagcagca gtgcgcctct gctcccttca gagcacagcc tggtgtcaag gtccaggttc 60
caccggctgc tgctgtcacc gcaggggagt ctagccccta ccagaaggag acacagaaga 120
atggccatct caactggttt gttcctgctg ctggggctcc ttggccagcc ctgggcaggg 180
gctgctgctg attcacaggc tgtggtgtgc gaggggactg cctgctatac agcccattgg 240
ggcaagctga gtgccgctga agcccagcat cgctgcaatg agaatggagg caatcttgcc 300
accgtgaaga gtgaggagga ggcccggcat gttcagcaag ccctgactca gctcctgaag 360
accaaggcac ccttggaagc aaagatgggc aaattctgga tcgggctcca gcgagagaag 420
ggcaactgta cgtaccatga tttgccaatg aggggcttca gctgggtggg tggtggagag 480
gacacagctt attcaaactg gtacaaagcc agcaagagct cctgtatctt taaacgctgt 540
gtgtccctca tactggacct gtccttgaca cctcacccca gccatctgcc caagtggcat 600
gagagtccct gtgggacccc cgaagctcca ggtaacagca ttgaaggttt cctgtgcaag 660
ttcaacttca aaggcatgtg taggccactg gcgctgggtg gtccagggcg ggtgacctat 720
accacccctt tccaggccac tacctcctct ctggaggctg tgccttttgc ctctgtagcc 780
aatgtagctt gtggggatga agctaagagt gaaacccact atttcctatg caatgaaaag 840
actccaggaa tatttcactg gggcagctca ggcccactct gtgtcagccc caagtttggt 900
tgcagtttca acaacggggg ctgccagcag gattgcttcg aaggtggcga tggctccttc 960
cgctgcggct gccggcctgg atttcgactg ctggatgatc tagtaacttg tgcctccagg 1020
aacccctgca gctcaaaccc atgcacagga ggtggcatgt gccattctgt accactcagt 1080
gaaaactaca cttgccgttg tcccagcggc taccagctgg actctagcca agtgcactgt 1140
gtggatatag atgagtgcca ggactccccc tgtgcccagg attgtgtcaa cactctaggg 1200
agcttccact gtgaatgttg ggttggttac caacccagtg gccccaagga agaggcctgt 1260
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gatggctctt tctactgctc ctgtaaagag ggctatattg tgtctgggga agacagtacc 1380
cagtgtgagg atatagatga gtgttcggac gcaaggggca atccatgtga ttccctgtgc 1440
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ggggtctttt gtagcagggg cactgtgttt tctgaactac cagccaggcc tccccaaaag 1560
gaagacaacg atgacagaaa ggagagtact atgcctccta ctgaaatgcc cagttctcct 1620
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tctgtgtctg cacacagtga caccgatggg cagaacctgc ttctgtttta catcctgggg 1860
acggtggtgg ccatctcact cttgctggtg ctggccctag ggattctcat ttatcataaa 1920
cggagagcca agaaggagga gataaaagag aagaagcctc agaatgcagc cgacagctat 1980
tcctgggttc cagagcgagc agagagccaa gccccggaga atcagtacag cccaacacca 2040
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cttcttagat gaggggggaag ccacatcatt ctgaatgact tgactggact ctcagcaaaa 2160
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gcctttgata tgtctggggg tgaaagctgt gtgttggttt gtcattgtgg ggagttttgt 2280
ggatattgac agacctcact caaacaccct tttcaaatcc aatagcaact ggttcctctg 2340
gttcctaatt agggggaaag gagtcagagg ggtgggacag ggtgggggga tggggcttca 2400
aagtttttc ttatcacttg atttatcatc gaaggagtta ctggtgctaa ttacaatgga 2460
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ttttaaaata tgtgcgtttg aaatctgttt catgcatcct ggaactgtgg gatgctcagg 2880
caagagtgac tttagtcttt cagtgaatgt tgcccagaat gtgggtaggg aaggctcaca 2940
ggttactctc ctccttagag ctacaacata acattctgag gggagtcaca gggttgcctt 3000
taaaaagtgg gagctatgtc atgctttgag ctttctgtta agcacctctc ctaataaact 3060
ctgaaaaaat aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 3120
a 3121

<210> 73
<211> 644
<212> PRT
<213> Homo sapiens

<400> 73
Met Ala Ile Ser Thr Gly Leu Phe Leu Leu Leu Gly Leu Leu Gly Gln
1 5 10 15

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Pro Trp Ala Gly Ala Ala Ala Asp Ser Gln Ala Val Val Cys Gly Gly
            20                  25                  30

Thr Ala Cys Tyr Thr Ala His Trp Gly Lys Leu Ser Ala Ala Glu Ala
        35                  40                  45

Gln His Arg Cys Asn Glu Asn Gly Gly Asn Leu Ala Thr Val Lys Ser
    50                  55                  60

Glu Glu Glu Ala Arg His Val Gln Gln Ala Leu Thr Gln Leu Leu Lys
65                  70                  75                  80

Thr Lys Ala Pro Leu Glu Ala Lys Met Gly Lys Phe Trp Ile Gly Leu
                85                  90                  95

Gln Arg Glu Lys Gly Asn Cys Thr Tyr His Asp Leu Pro Met Arg Gly
            100                 105                 110

Phe Ser Trp Val Gly Gly Gly Glu Asp Thr Ala Tyr Ser Asn Trp Tyr
        115                 120                 125

Lys Ala Ser Lys Ser Ser Cys Ile Phe Lys Arg Cys Val Ser Leu Ile
    130                 135                 140

Leu Asp Leu Ser Leu Thr Pro His Pro Ser His Leu Pro Lys Trp His
145                 150                 155                 160

Glu Ser Pro Cys Gly Thr Pro Glu Ala Pro Gly Asn Ser Ile Glu Gly
                165                 170                 175

Phe Leu Cys Lys Phe Asn Phe Lys Gly Met Cys Arg Pro Leu Ala Leu
            180                 185                 190

Gly Gly Pro Gly Arg Val Thr Tyr Thr Thr Pro Phe Gln Ala Thr Thr
        195                 200                 205

Ser Ser Leu Glu Ala Val Pro Phe Ala Ser Val Ala Asn Val Ala Cys
    210                 215                 220

Gly Asp Glu Ala Lys Ser Glu Thr His Tyr Phe Leu Cys Asn Glu Lys
225                 230                 235                 240

Thr Pro Gly Ile Phe His Trp Gly Ser Ser Gly Pro Leu Cys Val Ser
                245                 250                 255

Pro Lys Phe Gly Cys Ser Phe Asn Asn Gly Gly Cys Gln Gln Asp Cys
            260                 265                 270

Phe Glu Gly Gly Asp Gly Ser Phe Arg Cys Gly Cys Arg Pro Gly Phe
        275                 280                 285

Arg Leu Leu Asp Asp Leu Val Thr Cys Ala Ser Arg Asn Pro Cys Ser
    290                 295                 300

Ser Asn Pro Cys Thr Gly Gly Gly Met Cys His Ser Val Pro Leu Ser
305                 310                 315                 320

Glu Asn Tyr Thr Cys Arg Cys Pro Ser Gly Tyr Gln Leu Asp Ser Ser
                325                 330                 335

Gln Val His Cys Val Asp Ile Asp Glu Cys Gln Asp Ser Pro Cys Ala
            340                 345                 350

Gln Asp Cys Val Asn Thr Leu Gly Ser Phe His Cys Glu Cys Trp Val
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        355                 360                 365

Gly Tyr Gln Pro Ser Gly Pro Lys Glu Glu Ala Cys Glu Asp Val Asp
    370                 375                 380

Glu Cys Ala Ala Ala Asn Ser Pro Cys Ala Gln Gly Cys Ile Asn Thr
385                 390                 395                 400

Asp Gly Ser Phe Tyr Cys Ser Cys Lys Glu Gly Tyr Ile Val Ser Gly
                405                 410                 415

Glu Asp Ser Thr Gln Cys Glu Asp Ile Asp Glu Cys Ser Asp Ala Arg
            420                 425                 430

Gly Asn Pro Cys Asp Ser Leu Cys Phe Asn Thr Asp Gly Ser Phe Arg
        435                 440                 445

Cys Gly Cys Pro Pro Gly Trp Glu Leu Ala Pro Asn Gly Val Phe Cys
    450                 455                 460

Ser Arg Gly Thr Val Phe Ser Glu Leu Pro Ala Arg Pro Pro Gln Lys
465                 470                 475                 480

Glu Asp Asn Asp Asp Arg Lys Glu Ser Thr Met Pro Pro Thr Glu Met
                485                 490                 495

Pro Ser Ser Pro Ser Gly Ser Lys Asp Val Ser Asn Arg Ala Gln Thr
            500                 505                 510

Thr Gly Leu Phe Val Gln Ser Asp Ile Pro Thr Ala Ser Val Pro Leu
        515                 520                 525

Glu Ile Glu Ile Pro Ser Glu Val Ser Asp Val Trp Phe Glu Leu Gly
    530                 535                 540

Thr Tyr Leu Pro Thr Thr Ser Gly His Ser Lys Pro Thr His Glu Asp
545                 550                 555                 560

Ser Val Ser Ala His Ser Asp Thr Asp Gly Gln Asn Leu Leu Leu Phe
                565                 570                 575

Tyr Ile Leu Gly Thr Val Val Ala Ile Ser Leu Leu Leu Val Leu Ala
            580                 585                 590

Leu Gly Ile Leu Ile Tyr His Lys Arg Arg Ala Lys Lys Glu Glu Ile
        595                 600                 605

Lys Glu Lys Lys Pro Gln Asn Ala Ala Asp Ser Tyr Ser Trp Val Pro
    610                 615                 620

Glu Arg Ala Glu Ser Gln Ala Pro Glu Asn Gln Tyr Ser Pro Thr Pro
625                 630                 635                 640

Gly Thr Asp Cys
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<210> 74
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 74
tggtgtcgac gcagagtagc ggccgct 27

<210> 75
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 75
ggcccgggcc ggcc 14

<210> 76
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 76
tcgaggccgc ccgggcc 17

<210> 77
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 77
cagccctcac tccttctc 18

<210> 78
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 78
ggtggggtct ttcattcc 18

<210> 79
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 79
ttcagcaagc cctgactc 18

<210> 80
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 80
gccaccttcg aagcaatc 18

<210> 81
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 81
gagcggtaca ggagaatg 18

<210> 82
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 82
gcccacccaa ccaaatca 18

<210> 83
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 83
accgcgttct tctgtaac 18

<210> 84
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 84
cagctaacag caggatcc 18

<210> 85
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 85
gacccaggct gcccc 15

<210> 86
<211> 15
<212> DNA

<213> Artificial S ence

<220>
<223> Description of Artificial Sequence: Primer

<400> 86
ggtactcttg ttgag 15

<210> 87
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 87
gagggtacca agcttcgtgg tccgcaacca gaag 34

<210> 88
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 88
ctcagatctg aattcggcct gtccctcggt gaggag 36

<210> 89
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 89
gacaagatct cagctgaata gcgacccacc ctgtg 35

<210> 90
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 90
gcatctagag cggccgctca ggcctgtccc tcggtgagga g 41

<210> 91
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 91
gcatctagag cggccgcgaa cgctgcttag atctcct 37

<210> 92

<211> 131
<212> PRT
<213> Mus musculus

<400> 92
Glu Val Phe Leu Asn Pro Leu Gly Arg Asp Leu Leu Ser Ile Ser Asp
1 5 10 15

Gly Arg Xaa Arg Ala Ile His Arg Arg Gly His Asn Asp Gly Glu Asp
20 25 30

Ser Leu Thr His Thr Asp Val Ser Ser Phe Gln Thr Met Asp Gln Met
35 40 45

Val Ser Asn Met Arg Asn Tyr Met Gln Lys Leu Glu Arg Asn Thr Gly
50 55 60

Gln Leu Ser Val Asp Pro Asn Gly His Ser Phe Cys Ser Ser Ser Val
65 70 75 80

Met Thr Tyr Ser Lys Ile Gly Asp Glu Pro Pro Lys Val Phe His Ala
85 90 95

Ser Thr Gln Thr Arg Arg Ala Pro Gly Arg Ile Lys Glu Thr Arg Lys
100 105 110

Ala Met Arg Asp Ser Asp Ser Gly Thr Arg Lys His Gly Tyr Trp Ser
115 120 125

Ser Tyr Pro
130

<210> 93
<211> 131
<212> PRT
<213> Mus musculus

<400> 93
Arg Ser Phe Ser Glu Pro Phe Gly Arg Asp Leu Leu Ser Ile Ser Asp
1 5 10 15

Gly Arg Gly Arg Ala His Asn Arg Arg Gly His Asn Asp Gly Glu Asp
20 25 30

Ser Leu Thr His Thr Asp Val Ser Ser Phe Gln Thr Met Asp Gln Met
35 40 45

Val Ser Asn Met Arg Asn Tyr Met Gln Lys Leu Glu Arg Asn Phe Gly
50 55 60

Gln Leu Ser Val Asp Pro Asn Gly His Ser Phe Cys Ser Ser Ser Val
65 70 75 80

Met Thr Tyr Ser Lys Ile Gly Asp Glu Pro Pro Lys Val Phe Gln Ala
85 90 95

Ser Thr Gln Thr Arg Arg Ala Pro Gly Gly Ile Lys Glu Thr Arg Lys
100 105 110

Ala Met Arg Asp Ser Asp Ser Gly Leu Glu Lys Met Ala Ile Gly His
115 120 125

His Ile His

130

<210> 94
<211> 159
<212> PRT
<213> Mus musculus

<400> 94
Ala Lys Tyr Val Cys Leu Ala Asn Lys Asn Cys Pro Val Asp Lys Arg
1 5 10 15

Arg Arg Asn Arg Cys Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val
20 25 30

Gly Met Val Lys Glu Xaa Val Arg Thr Asp Ser Leu Lys Gly Arg Arg
35 40 45

Gly Arg Leu Pro Ser Lys Pro Lys Ser Pro Gln Glu Pro Ser Pro Pro
50 55 60

Ser Pro Pro Val Ser Leu Ile Ser Ala Leu Val Arg Ala His Val Asp
65 70 75 80

Ser Asn Pro Ala Met Thr Ser Leu Asp Tyr Ser Arg Phe Gln Ala Asn
85 90 95

Pro Asp Tyr Gln Met Ser Gly Asp Asp Thr Gln His Ile Gln Gln Phe
100 105 110

Tyr Asp Leu Leu Thr Gly Ser Met Glu Ile Ile Arg Gly Trp Ala Xaa
115 120 125

Xaa Ile Pro Gly Phe Ala Asp Leu Pro Lys Ala Asp Gln Asp Leu Leu
130 135 140

Phe Glu Ser Ala Phe Leu Glu Leu Phe Val Leu Arg Leu Ala Tyr
145 150 155

<210> 95
<211> 140
<212> PRT
<213> Mus musculus

<400> 95
Ser Gln Thr Arg Gln Gln Gly Pro Leu Arg Ser Ile Met Lys Asp Leu
1 5 10 15

His Ser Asp Asp Asn Glu Glu Glu Ser Asp Glu Val Glu Asp Asn Asp
20 25 30

Asn Asp Ser Glu Met Glu Arg Pro Val Asn Arg Gly Gly Ser Arg Ser
35 40 45

Arg Arg Val Ser Leu Ser Asp Gly Ser Asp Ser Glu Ser Ser Ser Ala
50 55 60

Ser Ser Pro Leu His His Glu Pro Pro Pro Pro Leu Leu Lys Thr Asn
65 70 75 80

Asn Asn Gln Ile Leu Glu Val Lys Ser Pro Ile Lys Gln Ser Lys Ser
85 90 95

```
Asp Lys Gln Ile Ly.  sn Gly Glu Cys Asp Lys Ala Tyr Leu .   Glu
            100                 105                 110

Leu Val Glu Leu His Arg Arg Leu Met Thr Leu Arg Glu Arg His Ile
        115                 120                 125

Leu Gln Gln Ile Val Asn Leu Ile Glu Glu Thr Gly
    130                 135                 140


<210> 96
<211> 652
<212> PRT
<213> Mus musculus

<400> 96
Met Ala Thr Ser Met Gly Leu Leu Leu Leu Leu Leu Leu Leu Leu Thr
  1               5                  10                  15

Gln Pro Gly Ala Gly Thr Gly Ala Asp Thr Glu Ala Val Val Cys Val
            20                  25                  30

Gly Thr Ala Cys Tyr Thr Ala His Ser Gly Lys Leu Ser Ala Ala Glu
        35                  40                  45

Ala Gln Asn His Cys Asn Gln Asn Gly Gly Asn Leu Ala Thr Val Lys
     50                  55                  60

Ser Lys Glu Glu Ala Gln His Val Gln Arg Val Leu Ala Gln Leu Leu
 65                  70                  75                  80

Arg Arg Glu Ala Ala Leu Thr Ala Arg Met Ser Lys Phe Trp Ile Gly
                85                  90                  95

Leu Gln Arg Glu Lys Gly Lys Cys Leu Asp Pro Ser Leu Pro Leu Lys
            100                 105                 110

Gly Phe Ser Trp Val Gly Gly Gly Glu Asp Thr Pro Tyr Ser Asn Trp
        115                 120                 125

His Lys Glu Leu Arg Asn Ser Cys Ile Ser Lys Arg Cys Val Ser Leu
    130                 135                 140

Leu Leu Asp Leu Ser Gln Pro Leu Leu Pro Asn Arg Leu Pro Lys Trp
145                 150                 155                 160

Ser Glu Gly Pro Cys Gly Ser Pro Gly Ser Pro Gly Ser Asn Ile Glu
                165                 170                 175

Gly Phe Val Cys Lys Phe Ser Phe Lys Gly Met Cys Arg Pro Leu Ala
            180                 185                 190

Leu Gly Gly Pro Gly Gln Val Thr Tyr Thr Thr Pro Phe Gln Thr Thr
        195                 200                 205

Ser Ser Ser Leu Glu Ala Val Pro Phe Ala Ser Ala Ala Asn Val Ala
    210                 215                 220

Cys Gly Glu Gly Asp Lys Asp Glu Thr Gln Ser His Tyr Phe Leu Cys
225                 230                 235                 240

Lys Glu Lys Ala Pro Asp Val Phe Asp Trp Gly Ser Ser Gly Pro Leu
                245                 250                 255
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Cys Val Ser Pro Ly.  yr Gly Cys Asn Phe Asn Asn Gly Gly  His
            260                 265                 270

Gln Asp Cys Phe Glu Gly Gly Asp Gly Ser Phe Leu Cys Gly Cys Arg
        275                 280                 285

Pro Gly Arg Arg Leu Leu Asp Asp Leu Val Thr Cys Ala Ser Arg Asn
    290                 295                 300

Pro Cys Ser Ser Ser Pro Cys Arg Gly Gly Ala Thr Cys Val Leu Gly
305                 310                 315                 320

Pro His Gly Lys Asn Tyr Thr Cys Arg Cys Pro Gln Gly Tyr Gln Leu
                325                 330                 335

Asp Ser Ser Gln Leu Asp Cys Val Asp Val Asp Glu Cys Gln Asp Ser
            340                 345                 350

Pro Cys Ala Gln Glu Cys Val Asn Thr Pro Gly Gly Phe Arg Cys Glu
        355                 360                 365

Cys Trp Val Gly Tyr Glu Pro Gly Gly Pro Gly Glu Gly Ala Cys Gln
    370                 375                 380

Asp Val Asp Glu Cys Ala Leu Gly Arg Ser Pro Cys Ala Gln Gly Cys
385                 390                 395                 400

Thr Asn Thr Asp Gly Ser Phe His Cys Ser Cys Glu Glu Gly Tyr Val
                405                 410                 415

Leu Ala Gly Glu Asp Gly Thr Gln Cys Gln Asp Val Asp Glu Cys Val
            420                 425                 430

Gly Pro Gly Gly Pro Leu Cys Asp Ser Leu Cys Phe Asn Thr Gln Gly
        435                 440                 445

Ser Phe His Cys Gly Cys Leu Pro Gly Trp Val Leu Ala Pro Asn Gly
    450                 455                 460

Val Ser Cys Thr Met Gly Pro Val Ser Leu Gly Pro Pro Ser Gly Pro
465                 470                 475                 480

Pro Asp Glu Glu Asp Lys Gly Glu Lys Glu Gly Ser Thr Val Pro Arg
                485                 490                 495

Ala Ala Thr Ala Ser Pro Thr Arg Gly Pro Glu Gly Thr Pro Lys Ala
            500                 505                 510

Thr Pro Thr Thr Ser Arg Pro Ser Leu Ser Ser Asp Ala Pro Ile Thr
        515                 520                 525

Ser Ala Pro Leu Lys Met Leu Ala Pro Ser Gly Ser Ser Gly Val Trp
    530                 535                 540

Arg Glu Pro Ser Ile His His Ala Thr Ala Ala Ser Gly Pro Gln Glu
545                 550                 555                 560

Pro Ala Gly Gly Asp Ser Ser Val Ala Thr Gln Asn Asn Asp Gly Thr
                565                 570                 575

Asp Gly Gln Lys Leu Leu Leu Phe Tyr Ile Leu Gly Thr Val Val Ala
            580                 585                 590

Ile Leu Leu Leu Leu Ala Leu Ala Leu Gly Leu Leu Val Tyr Arg Lys
```

595 600 605

Arg Arg Ala Lys Arg Glu Glu Lys Lys Glu Lys Lys Pro Gln Asn Ala
610 615 620

Ala Asp Ser Tyr Ser Trp Val Pro Glu Arg Ala Glu Ser Arg Ala Met
625 630 635 640

Glu Asn Gln Tyr Ser Pro Thr Pro Gly Thr Asp Cys
645 650

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/12, 1/21, 5/10, C07K 14/47, 16/18, A61K 38/17, G01N 33/50, C12Q 1/68

A3

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Filed on 21 August 1998 (21.08.98)

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(74) Agents: YAMIN, Michael, A. et al.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(88) Date of publication of the international search report:
22 June 2000 (22.06.00)

(54) Title: GENES THAT REGULATE HEMATOPOIETIC BLOOD FORMING STEM CELLS AND USES THEREOF

(57) Abstract

The present invention provides an isolated nucleic acid derived from an isolated hematopoietic stem cell. The present invention additionally provides an isolated hematopoietic stem cell specific protein or a portion thereof encoded by the provided nucleic acid. In addition, the present invention provides a method for generating a stem cell/progenitor cell from a primitive hematopoietic cell. Also, the present invention further provides a method for identifying the presence in a sample of a compound that modulates hematopoietic stem cell activity. The present invention additionally provides a molecularly defined primitive hematopoietic stem cell. Finally, the present invention provides a method of ex vivo expansion of hematopoietic stem cells.

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INTERNATIONAL SEARCH REPORT

Interna ial Application No
PCT/US 99/19052

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N1/21 C12N5/10 C07K14/47 C07K16/18
A61K38/17 G01N33/50 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE GENBANK [Online] Accession No. AA154742, 11 December 1996 (1996-12-11) MARRA M.: "EST; mouse lymph node cDNA clone IMAGE:634660 similar to B-cell activation protein BL34" XP002126589 compare nt 1-390 with nt 13-400 of seq. ID 1. ---	1-5, 7-20, 22-31, 42-50
A	HONG J.X. ET AL.: "Isolation and characterization of a novel B-cell activation gene." J. IMMUNOL., vol. 150, no. 9, 1993, pages 3895-3904, XP002126588 the whole document --- -/--	1-5, 7-20, 22-31, 42-50

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1999	31.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 99/19052

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MATTHEWS W ET AL: "A RECEPTOR TYROSINE KINASE SPECIFIC TO HEMATOPOIETIC STEM AND PROGENITOR CELL-ENRICHED POPULATIONS" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 65, no. 7, page 1143-1152 XP000615979 ISSN: 0092-8674 cited in the application	1-5, 7-20, 22-31, 42-50

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US 99/ 19052

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 46-49 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheets

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

claims 1-5,7-20,22-31,42-50)- partial

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 31,42,43,46,50 refer to modulators of the polypeptides without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclsed and supported (Art. 5 and 6) PCT. No search can be performed for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

Claims 18-19 are unclear with regard to their dependence from claim 16 and to their scope.

Claims 2,10,44 are inconsistent, in that Seq. ID 42 defines an amino acid sequence and not a nucleic acid.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1-5,7-20,22-31,42-50) - partial

An isolated nucleic acid derived from an isolated hematopoietic stem cell, expressed in and specific to a hematopoietic stem cell.

Said nucleic acid capable of hybridizing to seq. ID 1.

Corresponding vectors, host systems, polypeptides, antibodies and antobody-producing cells, detection methods, modulators and methods to identify them, therapeutic and pharmecautic applications.

2. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 1.

3. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 3

4. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 4

5. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 5

6. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 6

7. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 7

8. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 8

9. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 9

10. Claims: (1-5,7-20,22-31,42-50) - partial

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject matter 1, but limited to seq. ID 10

11. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 11

12. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 12

13. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 13

14. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 14

15. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 15

16. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 16

17. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 17

18. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 18

19. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 19

20. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 20

21. Claims: (1-5,7-20,22-31,42-50) - partial

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject matter 1, but limited to seq. ID 21

22. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 22

23. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 23

24. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 24

25. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 25

26. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 26

27. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 27

28. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 28

29. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 29

30. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 30

31. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 31

32. Claims: (1-5,7-20,22-31,42-50) - partial

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject matter 1, but limited to seq. ID 32

33. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 33

34. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 34

35. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 35

36. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 36

37. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 37

38. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 38

39. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 39

40. Claims: (1-5,7-20,22-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 40

41. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 41 (and for the polypeptide, seq. ID 42)

42. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 43 (and for the polypeptide, seq. ID 44)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

43. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 45 (and for the polypeptide, seq. ID 46)

44. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 47 (and for the polypeptide, seq. ID 48)

45. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 49 (and for the polypeptide, seq. ID 50)

46. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 51 (and for the polypeptide, seq. ID 52)

47. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 53 (and for the polypeptide, seq. ID 54)

48. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 55 (and for the polypeptide, seq. ID 56)

49. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 57 (and for the polypeptide, seq. ID 58)

50. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 59 (and for the polypeptide, seq. ID 60)

51. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 61 (and for the polypeptide, seq. ID 62)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

52. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 63 (and for the polypeptide, seq. ID 64)

53. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 65 (and for the polypeptide, seq. ID 66)

54. Claims: (1-5,7-31,42-50) - partial

Idem as subject matter 1, but limited to seq. ID 67 (and for the polypeptide, seq. ID 68)

55. Claims: (17,21) - partial

An isolated hematopoietic stem cell-specific protein or portion thereof.

Said protein characterized by seq. ID 70

56. Claims: (17,21) - partial

Idem as subject matter 56, but limited to seq. ID 71

57. Claims: (1-5,7-20,22-31,42-50) - partial; (6) - complete

Idem as subject matter 1, but limited to seq. ID 72 (and for the polypeptide, seq. ID 73)

58. Claims: (32-41) - complete

A method for identifying primitive hematopoietic stem cell-specific nucleic acids.